STUDIES

FROM

THE INSTITUTE FOR MEDICAL RESEARCH,

FEDERATED MALAY STATES.

No. 12.

THE ETIOLOGY

OF

BERI-BERI

BY

HENRY FRASER, M.D. (ABER.),

Director, Institute for Medical Research,

AND

A. T. STANTON, M.D. (TOR.),

Bacteriologist, Institute for Medical Research.

Singapore:

KELLY & WALSH, LIMITED, PRINTERS,

AND AT

SHANGHAI, HONGKONG AND YOKOHAMA.

1911.

Price: Seven Shillings.

THE ETIOLOGY OF BERI-BERI.

STUDIES

FROM

THE INSTITUTE FOR MEDICAL RESEARCH,

FEDERATED MALAY STATES.

No. 12.

THE ETIOLOGY

OF

BERI-BERI

BY

HENRY FRASER, M.D. (ABER.),

Director, Institute for Medical Research.

AND

A. T. STANTON, M.D., (TOR.)

Bacteriologist, Institute for Medical Research.

Singapore:

KELLY & WALSH, LIMITED, PRINTERS,

AND AT

SHANGHAI, HONGKONG AND YOKOHAMA.

1911.

Price: Seven Shillings.

PREFACE.

The inquiry, the results of which are submitted in the accompanying report, was begun under instructions from the Government of the Federated Malay States and we take this opportunity of recording our appreciation of the support extended by His Excellency the High Commissioner and other officials in furthering the interests of the research.

To Dr. G. Grijns of Weltevreden, Java, and to Dr. W. L. Braddon of Seremban, whose masterly researches on the etiology of Beri-beri formed the foundations on which we have attempted to build, as well as to many other fellow workers we are indebted for helpful criticism. For valuable assistance at various stages of the inquiry we have to thank our colleagues Dr. W. Fletcher, Mr. B. J. Eaton and Mr. J. R. Hill.

H. F.

A. T. S.

CONTENTS.

	PAGE.
Introduction	1
Rice—histological and chemical	17
Feeding experiments on animals	28
The Poison hypothesis	34
Experiments with various white rices	40
Starvation and forced feeding experiments	43
Experiments with unpolished rices	45
Experiments with rice meal or polishings	49
The Phosphorus content of rices	51
The effect on protective substances of exposure to high temperatures	63
Experiments to determine the nature of the protective substances	66
Conclusions	85
References	88

THE ETIOLOGY OF BERI-BERI.

THE etiology of beri-beri has been the subject of investigation by workers in many countries, and in view of the wide divergence of the conclusions arrived at and the varying results of the preventive measures suggested, it will be apparent that the problem is one of great complexity. Not a little of this confusion has been introduced by the lack of agreement among authorities as to what disease conditions are to be included under the name beri-beri. A survey of the voluminous literature which concerns itself with this disease will show not only that forms of polyneuritis of different etiology have been called beri-beri, but that even diseases of which polyneuritis may not be a prominent feature such as "epidemic dropsy" "ship beri-beri" "Ceylon beri-beri" and the like have been included under this name. It is not to be expected therefore that any single etiological factor will satisfactorily explain all the recorded outbreaks of so-called beri-beri.

The Malay Peninsula has long been known as an "endemic focus" of beri-beri and the recorded sickness and mortality rates from that disease during the past several decades show that great economic losses have resulted from its ravages. The Government of the Federated Malay States have in consequence greatly interested themselves in furthering investigation into the cause of beri-beri and five years ago the task was assigned to us of attempting to define more narrowly the etiological factors concerned. In previous papers the results of this work as obtained from time to time have been set forth and it is now proposed to review the progress made and to submit the details of the work upon which our conclusions are based.

At the time this inquiry was begun, students of the disease were divided into two principal groups in regard to their views of its origin. On the one hand it was held that beri-beri had its origin in some organism, bacterial or protozoal, and that the disease was communicable, directly or indirectly, from man to man (Manson, Scheube, Daniels, Wright); on the other hand it was maintained, more particularly by physicians in Eastern Asia where the disease is most common, that the cause of beri-beri was to be sought for in food.

An examination of the evidence put forward by these latter workers, notably Takaki in Japan, Eijkman, Grijns, Vorderman and others in the Dutch East Indies and Braddon in this country made it difficult not to believe that the question of diet was a factor of great importance in the causation of beri-beri and that inquiry along this line held out the best prospect of success.

Attention was directed long ago to the influence of diet in the causation of beri-beri. Wernich (1) who studied the disease in Japan writing in 1878 says: "The kak-ké is a chronic constitutional disorder of blood-making and of the vascular system. Rice as the exclusive food of the people is answerable for it in a quite especial way. Not, however, as some have thought because it is used in a decomposed state, but because it is used in such quantities that the power of assimilation is gradually lost for other kinds of food; and even the large quantity of rice is unable to render the nutrition and blood-making adequate."

Van Leent (2) from his experiences in the Dutch East Indies wrote: "The dietetic error which I regard as the one and only cause of the morbid composition of the blood in beri-beri consists in the too small proportion of albuminous substances and fat." In support of this he gives the experiences of the disease in the Dutch East Indian fleet from 1870 to 1878 particularly during the war in Acheen both among the native and European members of the crews.

Takaki (3) believed the disease to be due essentially to nitrogen starvation and in 1884 introduced certain reforms in the diet of the Japanese Navy. Up to this time beri-beri had been an important source of invaliding and mortality among the sailors, of whom about 30% were incapacitated annually from that disease. By the beginning of 1890 under the improved diet beri-beri was wholly eradicated and the incidence of other diseases greatly decreased. Takaki still adheres to this view of beri-beri causation and in the course of a series of lectures on "The Health of the Japanese Navy and Army" in 1906 (4) he gives fresh evidence in its support: "Another illustration of the influence of diet upon the health of the men is shown by the fact that there was not one case of beri-beri among the sailors of the naval brigade before Port Arthur although there was a large number of cases in the army. These men lived among the soldiers and under exactly the same conditions but they differed from the soldiers in one respect that they were supplied with one pound of meat, ten ounces of barley, and twenty ounces of rice *per diem* while the soldiers were supplied with five ounces of meat and thirty ounces of rice. The above example confirms my view that beri-beri largely occurs among men who are fed with an insufficient quantity of nitrogenous food and and excess of carbohydrates."

Durham (5) who studied the disease on Christmas Island and in the Malay Peninsula while concluding that: "so far as there was any semblance of a positive result in the observations it is suggestive that beri-beri is communicated from person to person more or less directly or through fomites as an actual infection" stated that "It was difficult not to believe that the presence of some constituent of the diet had a sheltering effect on the nervous system of the individual" and again that "it is suggested that certain articles of diet by virtue especially of containing phosphorized and fatty matters may tend to ward off the disease when given in sufficient amount."

Professor Chittenden quoted by Bryce ("British Medical Journal" Dec. 11, 1909) says with regard to the statement, that

the Japanese Navy had found that a high protein diet was accompanied by a diminution in the number of cases of beri-beri he contended that it did not necessarily follow that the increase of protein was the cause. He thought that it was much more likely that other elements were introduced into the food capable of accounting for the disappearance of the disease. Protein pure and simple is unlikely to be utilised as a food in the body. It is much more likely that it requires to become a salt of lime, potash or soda before it can be available for dietetic purposes. He thinks the mineral salts introduced with the protein prevented beri-beri much in the same way that lemon juice prevents the appearance of scurvy.

Epidemically and endemically the occurrence of beri-beri is certainly greatest in those tropical and subtropical countries whose inhabitants partake of a diet in which rice forms an important and generally the staple constituent. The other articles of diet vary with the country but rice varies merely in kind or quality and but little in regard to the quantity consumed.

In those countries where the disease is endemic the incidence has always been greatest among the poorer classes, inmates of public institutions and the like. Supporters of dietary hypotheses have therefore sought to account for the occurrence of the disease in that the diet consumed was deficient in some substance or substances essential for nutrition and those workers who have incriminated rice consider either that a diet in which this article bulks largely is deficient in protein or fat, or that the rice has become diseased from the action of moulds or other organisms.

The suggestion of a relationship between a rice diet and beri-beri is a very old one and there are numerous references to it in the literature of the disease. In the second edition of Hirsch's "Handbook of Geographical and Historical Pathology," published in 1881, a review is given of the various hypotheses propounded to explain the origin of beri-beri.

Dr. Hirsch says: "The conjecture that the morbid poison is some *specifically noxious thing in the food*, brought about by the local conditions, is a probable one, and there has been no lack of hypotheses in that sense. At an early period the question was propounded whether it might not be some *poisonous property of (decomposed) rice* that represented the cause of the malady."

This conjecture was revived by Braddon (6) who studied the disease in the Federated Malay States and the contributions made by this observer have led to material advances in our knowledge. Braddon was the first to show the closeness of the relationship which exists in this country between the consumption of white rice as a staple article of diet and the disease beri-beri. Of even greater importance was his demonstration of the fact that where the staple article of diet is rice which had been parboiled before husking as used by the Tamil population, or rice as prepared by the Kampong Malays, the disease does not occur.

As will presently appear this position which for many years Braddon defended in local medical circles has since been abundantly justified by controlled experiments.

Braddon's conception of the mechanism of beri-beri causation through white rice ("stale uncured rice") was that "the cause of the disorder is not indeed rice, *quâ* rice, or as an article of diet, but diseased rice; rice from which some poison derived from decay, due perhaps to some fungus, or mould, or germ, or spore originally perhaps growing upon the husk, has become mixed during the process of milling; or upon which such fungus may have grown and such poison have been produced after milling."

In accordance with this hypothesis Braddon recommended as preventive measures the use of fresh rice (Malay rice or freshly milled rice), or rice that had been parboiled before

husking. He conjectures that in the parboiling process, the hypothetical organisms are made to germinate and in that vulnerable stage are destroyed by heating. He compares the process to that employed in laboratories to sterilize or render free of noxious germs various materials which may contain them.

This hypothesis of beri-beri causation has not met with acceptance by the later investigators, Grijus, Kiewit de Jonge, Schaumann, Aron and others, who regard the disease as the result of some defect in the composition of the foodstuffs ingested.

With a view to determine the position of white rice in regard to the causation of beri-beri, Dr. Fletcher (7) carried out a series of observations extending over two years among the patients in the Lunatic Asylum, Kuala Lumpur. In his report of the results Dr. Fletcher says: "During the year 1905 a large number of lunatics in the Kuala Lumpur Lunatic Asylum suffered from beri-beri. Ninety-four of the two hundred and nineteen lunatics treated in the Asylum were affected and twenty-seven died from the disease.

"With the purpose of testing Dr. BRADDON'S theory, observations were carried on in the following years (1906, 1907) with regard to the diet of the patients. Half of the patients were placed on a diet of 'cured' (parboiled) rice while the other half remained on the diet of 'uncured' (white polished) rice which kind all the lunatics of the Asylum had been eating previous to the commencement of the observations."

"The lunatics were housed in two exactly similar buildings on opposite sides of a quadrangle surrounded by a high wall. On December 5, 1905, all the lunatics at that time in the hospital were drawn up in the dining shed and numbered off from the left. The odd numbers were subsequently domiciled in the ward on the east side of the courtyard and no alteration was made in the diet; they were still supplied with the same uncured rice as in 1905. The even numbers were quartered in the ward on the west side of the quadrangle and received the same rations as the occupants of the other ward, with the exception that they were supplied with 'cured' rice instead of the 'uncured' variety. The two batches of patients were kept in separate wards and fed at different times. Separate cooking and feeding utensils were used but otherwise the patients were allowed to associate together. At the half year the two batches were changed over to each others apartments."

The result for 1906 was that "out of 124 inmates fed on 'uncured' rice 34 suffered from beri-beri, 2 of whom were

suffering from the disease on admission and 18 died; whereas among 123 inmates fed on cured rice there were only 2 cases of beri-beri both of whom were suffering from the disease on admission, and no deaths."

At the end of 1906 there remained 35 lunatics in the "cured" rice ward and 30 in the "uncured" rice ward. The first patient admitted in 1907 was admitted to "uncured" rice, the second to "cured," the third to uncured and so on to the end of the year.

During the year 1907, 136 patients were treated in the "uncured" rice ward; of these patients 28 suffered from beri-beri, 4 of whom were suffering from the disease on their admission. During the same year 131 patients received a diet containing "cured" rice; four of them were admitted actually suffering from beri-beri but none of these 131 patients developed the disease in the Asylum.

Fletcher concludes that: "The cause of beri-beri is to be sought for in the diet. The result of the experiment tends to show that white polished rice, although of the best quality, is a cause of beri-beri acting either by some poison which it contains or by a starvation due to some defect in the nutritive value of such rice."

In 1907-1908 the writers carried out a series of observations designed to test the position of white rice as a causative agent in the production of beri-beri. A detailed account (8) of this inquiry has already been published and the results may here be summarized.

It was considered important that these observations should be made in a place hitherto free from the disease and where the operation of factors other than diet could be excluded or adequately controlled. Satisfactory conditions were obtained at Durien Tipus in Negri Sembilan where some three hundred Javanese labourers were engaged in the construction of a new road through virgin jungle, remote from the complex conditions which interfere with observations in populous areas. The quarters occupied by these labourers were new and the sanitary conditions were satisfactory.

The three hundred labourers were divided into two parties of approximately equal numbers and were housed some miles apart. Before beginning the experiment an examination was made of each person and the presence of cases of existing or recent beri-beri was thereby excluded.

To one party white rice (No. 2 Siam) was issued as the staple article of diet, and to the other party parboiled rice. In about three months cases of beri-beri began to occur among the members of the party on white rice. When a certain number of cases had been noted white rice was discontinued and thereafter no cases occurred. No sign of the disease appeared among the control party on parboiled rice.

The conditions were then reversed. The party hitherto on parboiled rice were given white rice and after a somewhat longer interval than in the first instance, beri-beri broke out in this group also. This outbreak ceased on discontinuing the issue of white rice. Again no sign of the disease appeared among

the control party on parboiled rice. By the transfer of individuals suffering from beri-beri and of whole groups in which the disease was occurring it was found possible to test the influence of place considered as a nidus of infection and also to test the possibility that the disease was communicable from one individual to another.

The average daily ration was as follows :—

Rice	21.3 oz.	603 grammes
Dried Salt Fish ...	4.25 ,,	120 ,,
Onions	1.75 ,,	50 ,,
Potatoes ...	1.75 ,,	50 ,,
Coconut oil ...	0.85 ,,	24 ,,
Coconut ...	1.50 ,,	42 ,,
Tea	0.12 ,,	3.4 ,,
Salt	0.1 ,,	2.8 ,,

In a recent publication Dr. H. Schaumann (9) has dealt with the composition of this diet and quotes the results in support of his view that the disease beri-beri is due to a diet defective in substances having a high organically-combined phosphorus content.

As Dr. Schaumann has assigned values to the various food-stuffs comprising this diet which differ from those given by us it would appear that he had not before him a copy of the publication in which these were furnished, namely No. 10 of this series published in 1909. Also as the authorship of the paper published in "The Lancet" (1909 No. 4459 page 451) is wrongly quoted throughout the text it may be assumed that Dr. Schaumann had not an opportunity of consulting it in the original. In these circumstances we venture to direct attention to some errors into which we believe Dr. Schaumann has fallen.

The analyses upon which our calculations were based were carried out with the actual foodstuffs employed, and were not taken upon the authority of a text book on dietetics. We feel called upon to make this explanation as a protest against the use

of analyses of foodstuffs given in text books, however eminent the authority, for the calculation of the relative values of diets. As we shall hope to show later, fundamental differences may exist in the nutritive value of various combinations of foodstuffs which are not revealed by the ordinary methods of analysis and there are factors in the composition of food which are of much more complex nature than its protein, fat and carbohydrate content or its calorific value.

Observations have shown that beri-beri is definitely associated with the continuous consumption of a diet of which white rice is the staple and must be due either to a poison contained in white rice or to a deficiency in such a diet of some element of high physiological value. If the latter, then accuracy is imperative and can only be attained by analysis of the actual foodstuffs eaten by those among whom the disease occurred. So far as we are aware such an opportunity has only been afforded by our planned observations extending over twelve months and which are now under discussion.

The various articles composing the diet issued were submitted to analysis in this Institute by Mr. B. J. Eaton.

The average of a considerable number of analyses was as follows:—

	Moisture.	Protein.	Fat.	Carbohydrate.	Ash.
	%	%	%	%	%
White rice	13.85	7.45	0.17	78.02	0.51
Parboiled rice ...	14.03	7.8	0.53	76.92	0.72
Dried salt fish ...	48.1	35.7	2.96		13.24
Onions	86.44	1.5	0.28	11.28	0.5
Potatoes	65.3	1.8	0.2	31.66	1.04
Coconut	45.3	3.95	34.6	15.2	0.95

Based on these analyses the diet issued to those on white rice as the staple was calculated to consist of:—

Protein.	Fats.	Carbohydrate.	Salts.
91.45 grammes	43.70 grammes	499.16 grammes	23.06 grammes.

The diet issued to those on parboiled rice was calculated to consist of:—

Protein.	Fats.	Carbohydrate.	Salts.
93.56 grammes	45.88 grammes	492.54 grammes	24.33 grammes.

Dealing with this diet Dr. Schaumann, on page 79 of his monograph, gives a table which shows, assuming white rice to contain 7.8 % Protein, that the diet received by those on white rice contained 103.7 grammes of Protein, whereas calculated from the analyses of the actual foodstuffs supplied this diet contained 91.45 grammes of Protein. Again on page 81, assuming parboiled rice to contain 9.05 % Protein he states that the diet of those on parboiled rice contained 111 grammes of Protein or 7.3 grammes *more* than that contained in the white rice diet whereas the fact is that the parboiled rice diet contained 93.56 grammes of Protein or 2.1 grammes more than that contained in the white rice diet.

Again on page 339 Dr. Schaumann compares the diet of those on parboiled rice with that of those on white rice. The analysis of the foodstuffs there given differ materially from the analysis of the actual foodstuffs issued. By his calculations Dr. Schaumann now shows that those consuming the parboiled rice diet receive *less* protein than those consuming the white rice diet, as he here takes it that parboiled rice contains less Protein than white rice (6.38 % Protein for parboiled rice and 7.03 % Protein for white rice) which is not in accord with the statement made previously by him.

On page 363, Table XII, Dr. Schaumann gives the composition of the various foodstuffs in respect of Protein, Fat, Carbohydrate, Fibre, Total Ash and Phosphorus Pentoxide, these he takes as the basis for his final calculations.

It is possible that a parboiled rice may be found which yields 2.08 % of fat and 3.57 % of ash but among the numerous specimens examined in this Institute we have not met with such a rice.

Whatever methods of analysis are employed it may well be that the defect of nutrition, which we regard as the cause of beri-beri, will escape observation but certainly it appears that when the actual foodstuffs issued in a diet are not the subject of analysis, the results are not likely to help materially towards a solution of the problem of beri-beri causation. The valuable work which Dr. Schaumann has accomplished in this domain of research would in our opinion have been greatly enhanced in value had the opportunity been available to him of studying the actual dietary conditions under which beri-beri arises.

It is generally accepted that the dietary requirements per kilogramme of body weight are Protein 2 grammes, Fat 1.5 grammes, Carbohydrate 6 grammes, Salt 0.5 gramme. The average body weight of the people under our observation was 100 lbs. nearly—the diet should therefore contain—

Protein.	Fat.	Carbohydrate.	Salt.
90 grammes	68 grammes	272 grammes	20 grammes

Comparison of this calculated standard diet with the diets consumed by the persons under observation will show that the issued diets cannot be regarded as deficient save in respect of fats. This latter deficiency is however more than compensated for by the excess of carbohydrates. With a sufficiency of protein in a diet, fats and carbohydrates are to a great extent interchangeable and the inhabitants of warm countries habitually consume less fat and more carbohydrate than do the inhabitants of cold countries.

It will be noted that the analyses of these foodstuffs did not include an estimation of the relative proportions of the inorganic salts composing the ash, nor did they take account of the manner of combination organic or inorganic, in which these substances originally existed in the rice grain. This matter will be referred to in some detail at a later stage of this report.

The conclusions arrived at as a result of this inquiry were stated as follows:—

1. In the course of a systematic inquiry especially undertaken to test the position of white rice as a causative

agent in beri-beri it was observed that twenty cases of this disease occurred among two hundred and twenty people on white rice who were continuously present in the various parties during the course of the outbreaks. In the parties on parboiled rice during the same periods and under similar conditions, among two hundred and seventy-three people no sign of the disease appeared.

2. Since all cases presenting doubtful signs of the disease were excluded we are of opinion that there were many other cases which in the ordinary routine of clinical practice would have been regarded as beri-beri. Such cases only occurred among people who consumed white rice and their inclusion would not strengthen the case for an infectious origin of the disease.

3. No case of beri-beri occurred in any person who had been on white rice for less than eighty-seven days.

4. Systematic examinations were made of the blood and urine of patients suffering from beri-beri. Various methods of examination were employed but in no case were organisms found other than those well-known as the causative agents of other diseases.

5. In the course of the inquiry patients in various stages of beri-beri were in contact with parties of men on parboiled rice. The results of observations made on such occasions furnished evidence that the disease is not a communicable one.

6. Removal of patients from the place where they had contracted the beri-beri did not influence the progress of the disease and the removal of entire parties from the place where the disease had occurred did not influence the progress of an outbreak so long as they continued on white rice.

These observations suggest that place *per se* or considered as a nidus of infection has no influence upon the development of beri-beri.

7. In three instances in which definite outbreaks of beri-beri occurred among parties of men on white rice, substitution of parboiled rice was followed by cessation of the outbreak.

8. The occurrence of beri-beri cannot be attributed to deficiency in the diet issued in respect of either protein. fat, carbohydrate or salts as estimated by the methods in common use.

9. No evidence was obtained to show that any article of the diet other than white rice was responsible for the occurrence of beri-beri.

10. Ankylostomes and other nematode worms were not found in a larger proportion of patients suffering from beri-beri than in the general population under observation.

11. The general results support the view that the disease beri-beri as it occurs in the Malay Peninsula has an intimate relationship with the consumption of white rice and further research along these lines is justified.

On the 26th of April 1909, Dr. J. D. Gimlette reported to the Senior Medical Officer, Selangor that a number of Malays recently enlisted into the Police Force and then stationed at the depôt Kuala Lumpur had reported themselves sick. On examination Dr. Gimlette had found that they were suffering from beri-beri. He adds "The occurrence is of interest because it has been possible to recognise the disease in an early stage. The majority of those attacked are newcomers who have recently fallen ill

while their rice (No. 2 Siam) has, I understand, only recently been supplied through a new sub-contractor. The patients are all Malays with one exception, a Javanese."

The rice was changed from white polished rice to parboiled rice on April 27th, and on June 2nd, Dr. Gimlette reported that the occurrence of the disease had ceased and that no deaths had occurred among those suffering from beri-beri.

Throughout the course of the inquiry at Durien Tipus samples of the white rice issued were collected daily and forwarded to the Institute.

Through the courtesy of Dr. Gimlette and Dr. Freer, Senior Medical Officer Selangor, we were enabled to obtain a bag of the white rice which was in use at the depôt at the time of the outbreak of beri-beri.

So far as the laboratory aspect of the question was concerned therefore an abundant supply of the rices which actually caused these outbreaks of beri-beri was secured. In connexion with the plan of work which we had formed we regard this circumstance as of the first importance. It could have led to no real advance in knowledge if analyses of a series of rices from various sources had been carried out without regard to their connexion with outbreaks of beri-beri. Having first shown that the consumption of a certain white rice was the cause of an outbreak of beri-beri we were in a position to proceed further and by analysis of and experiments with that rice to seek for the explanation of this relationship. The continuity of work thereby established we believe to be one of the principal merits of the observations here recorded.

Rice.

General.

The cultivation of rice has existed from ancient times. It is a cereal indigenous to certain areas in both hemispheres but its culture now extends over wide areas in tropical and subtropical countries possessing an assured and heavy rainfall; also with the aid of irrigation, cultivation has been extended to areas not possessed of a sufficient rainfall.

The operation of these factors has produced many species and varieties of rice so that their number is now very great. We are not in a position to treat this subject in a comprehensive way and must of necessity confine our remarks to the countries and their rices which have come under our immediate observation.

In the Straits Settlements and Federated Malay States a limited amount of rice is grown. The quantity is quite inadequate to supply the demand of a country which is rapidly and extensively being opened up and therefore supplies have to be imported from the great producing countries more especially Siam, Burma and French Indo-China.

Rice is derived from the fruit, botanically a caryopsis, of plants belonging to the genus Oryza.

The product as it reaches this country is in the form of unhusked fruit, the partially husked fruit, or the finished product rice.

The imported unhusked or partially husked grain is converted into rice in the mills of Penang and Singapore. So far as can be ascertained the principal reason for the importation of the unfinished product is in order that parboiled rice may be prepared locally and sold at a lower price than the parboiled rice imported from India. The local mills are of course adapted to the production of ordinary white rice and the question as to which variety of rice a mill shall produce is answered by the demand.

The indigenous natives (Malays) in the country districts grow the grain in quantities sufficient for their own requirements and from it prepare the rice by primitive methods of pounding and winnowing.

The oriental immigrants, natives of India, China and Java, come to this country either as workers on mines or on estates and therefore have not the opportunities for growing the rice they require. The immigrant Indians are for the most part recruited from the south of India and prefer parboiled rice, which must be cheap. It is to meet this demand that the large rice mills of Penang, Singapore and Perak have been called into existence. The natives of China and Java prefer a rice which has not been parboiled and for their requirements the grain is husked and polished by machinery. The product is commercially known as Rangoon, Siam or as we prefer to call it here white rice.

The kinds or forms of rice used in this country may therefore for purposes of this account be grouped under three divisions Parboiled rice, Native or Malay rice and white rice.

Parboiled Rice.

As prepared in the large power-mills the grain is soaked in water for 24—48 hours, the water is then run off and the grain is transferred to cylinders which are lightly covered and steam is passed through the contents for five or ten minutes. The grain is thereafter transferred to open paved courts and dried in the sun. The husk is now more readily detached than in the untreated grain but the contents of the grain have been rendered tough and semi-translucent.

In the milling of this parboiled grain the husk is removed and the rice is subjected to a limited amount of pearling or polishing in a machine provided with stone facings. By no process can such rice be made to appear white, consequently polishing is as a rule employed merely to complete the removal of fragments of husk and most of the pericarp.

Parboiled rice is also prepared on a less pretentious scale in small mills not provided with steam plant. The product is similar to that produced in the larger mills but in view of the insanitary conditions prevailing in many of these places, numbers of them have been compulsorily closed.

Parboiled rice prepared in this manner has a peculiar disagreeable penetrating odour caused by the preliminary soaking in cold water; by soaking the grain in hot water in place of cold water and for a shorter period, the occurrence of this disagreeable odour can be prevented.

Other manufacturers have sought to improve their product by subjecting the grain to more extensive polishing or pearling; as will be shown later this may be attended with dangerous results to those who consume such a highly-polished parboiled rice as the staple of their diet.

According to Hooper (10) rice in Bengal is treated before husking by three methods—

(1) Hot water is sprinkled over the padi.

(2) The padi is soaked in cold water for 24 hours.

(3) The padi is first soaked in water and afterwards it is boiled.

In each case the padi is thereafter dried in the sun or by other means. When the grains are sufficiently dry they are husked in a pestle and mortar.

Watt (11) states that "In India a large part of the rice sold in shops and exported to Europe as an article of food has been prepared by being first half boiled then dried in the sun and finally husked by the ordinary pestle and mortar. Such rice is in trade termed "parboiled." Husking without boiling is a very tedious process when done by hand."

According to the same authority, in India proper power mills for the preparation of rice are very few in number. In 1904 there

were in all India 127 rice mills with 17,814 employees and of this number 114 mills with 17,016 employees were located in Burma.

Parboiled rice prepared in India has no objectionable odour. It is imported into this country to a limited extent for consumption by the more affluent natives of India.

The objectionable odour possessed by the locally prepared parboiled rice has hitherto militated against the more widespread use of this kind of rice by others than natives of India and if the consumption of parboiled rice is to be encouraged such improvements in the process are required as will produce a rice similar to the parboiled product of India.

White rice.

In the milling of white rice in large power mills the padi is first deprived of the husk. This is done by passing it into a machine called the "huller" which consists of two iron discs faced with a cement of which emery is one of the ingredients. The padi enters in a stream at the centre and is driven by centrifugal action between the discs to the periphery. By this means the husk is cracked. The mixed rice and husk are now passed over the winnowing fans which blow away the husk.

The grain still covered with its brown, yellowish or other coloured pericarp now passes to a machine in which the whitening process takes place. This machine consists of a conical drum revolving at a high velocity; the drum is faced with emery cement and is surrounded by a casing lined with steel wirecloth. In the more modern mills the space between the cone and the wirecloth through which the rice passes can be altered by vertical adjustment of the spindle so that the necessary amount of attrition of the rice is secured and the desired amount of milling attained. In this process the fruit wall or pericarp, the layers subjacent to it (the subpericarpal layers) as well as the embryo are removed.

The whitened grain now passes to the polisher which consists of a revolving conical drum covered with strips of sheepskin, the whole being surrounded by a casing lined with wire-cloth. The polishing is accomplished by the rice passing down between the sheepskins and wirecloth.

In the milling and polishing machines the rice meal or "polishings" passes through the wire-cloth casings and is collected. Rice polishings are commonly used as a foodstuff for cattle and pigs. We are informed that the natives in the neighbourhood of the rice mills in Burma consume rice meal in the form of gruel.

White rice is graded commercially as Rangoon and Siam, also in accordance with the unbroken condition of the grains as No. 1 quality, or the grain mixed with broken grains as No. 2 quality and so on. Siam rice of the best quality is a long slender grain, almost white and free from dust. Rangoon rice of the best quality is a shorter and more plump grain, white and free from dust.

We should be glad if it could be made clear that in using these trade terms we do so merely as a convenience and with no intention to suggest that the padi produced in Indo-China or Siam is inferior to that produced elsewhere. Misapprehension has arisen in commercial circles as to the significance of these researches and the matter has even been the subject of correspondence between the various Governments. At the local Agri-Horticultural Exhibitions *Padi Siam* and *Padi Rangoon* grown locally are exhibited among a host of others. A certain type of grain in commerce has come to be associated with these names and in our use of the terms we do not mean to suggest that they necessarily indicate the country of origin of the rices used in our experiments. With the important questions of the nutritive values of different kinds of padi and the influence thereon of soil, climate, and methods of cultivation we have not concerned ourselves.

Malay or Native rice.

This is prepared from the grain grown by the Malays and the preparation of the rice is almost invariably carried out by the women who prepare it in quantities sufficient for the immediate wants of the household. The grain is sun-dried and transferred in suitable quantities to a wooden mortar fitted with a long wooden pestle. The grain is pounded until the husks are detached and these are removed by winnowing. By repeated pounding and winnowing the husk is entirely removed as well as the pericarp for the most part and the subjacent layers only to a limited extent by attrition and bruising.

The finished product is yellowish and is an admixture of broken and unbroken grains.

By repeated pounding and careful hand-picking it is possible to obtain a fairly white rice free from unbroken grains but the labour entailed is considerable and the natives are usually quite satisfied with a rice from which the entire husk and the most of the pericarp have been removed.

Histology.

In order to compare the different kinds of rice as to their histological characters and in order to examine rices from beri-beri outbreaks for the presence of organisms, it was necessary to obtain entire sections of the grain of sufficient thinness.

The following process was devised and found to yield suitable sections.

The grains are softened for a month or thereabouts in a mucilage composed of :—

Gum acacia	...	...	... 4 pts.
Solution of Carbolic Acid (1-20)			... 6 ,,

The softened grains are freed from excess of mucilage and imbedded in celloidin. The imbedded grains are placed in alcohol (60%) and after a few days are ready for cutting.

Sections were examined for moulds and for this purpose were stained by the following method :—

1. The sections were stained in anilin-gentian violet solution for from five to ten minutes.

2. The sections were freed from excess of stain and treated with Gram's iodine-solution for one minute.

3. The sections were freed from excess of iodine-solution and washed in absolute alcohol so long as stain continued to come away.

4. The sections were then treated with anilin oil for five minutes. After which the excess of oil was removed and the sections mounted in Canada balsam.

Plates I, II, and III are drawn from actual specimens and reproduce the colouration taken up by the various tissues by this method.

Numerous sections from various kinds of rice were examined in this way. In none of them were moulds or fungi recognised but the method produced excellent differentiation of the tissues and it was easily possible to understand and appreciate the histological differences between padi, parboiled rice, white rice and Malay rice.

A transverse section of a rice grain from which the paleae (husk) have been removed shows three zones (Plate I):—

1. The outer thin pericarp.

2. The layers subjacent to the pericarp, or subpericarpal layers, composed of cells filled with aleurone and fat and comparatively free from starch grains.

3. The remainder of the section, constituting the major part of the section and composed of cells filled with starch grains.

If sections of rice grain be treated with any of the ordinary stains for fat such as Osmic Acid, Sudan III, it will be seen that fat is practically confined to the second zone or subpericarpal layers; a few scattered oil globules may be recognized in the central or starch zone.

Section of a polished rice grain (Plate II) show that the pericarp and most of the subpericarpal layers have been removed. There is usually but a remnant of the fat containing layer and at times not even that remains, the section consisting almost entirely of the starch-containing cells.

Section of parboiled rice (Plate III) which has not been subjected to excessive pearling or polishing show the pericarp mostly removed but the subpericarpal layers practically unaffected.

Sections of Malay rice present an appearance similar to that presented by section of the grain free from husk but the pericarp is removed to a degree the extent of which depends on the amount of pounding and attrition to which the grains have been subjected.

Chemical.

In view of the accumulated mass of evidence which shows that the continuous consumption of rice from which the subpericarpal layers have been removed by polishing causes beriberi and in view of the histological differences demonstrated between polished and unpolished rices, the suggestion presented itself that by the removal of the subpericarpal layers the grain is deprived of some important nutritive substance or substances.

Analyses were made of the various kinds of rice and for comparison the average results of these analyses are given here :—

	Protein.	Fat.	Carbohydrate.	Ash.	Moisture.
	%	%	%	%	%
Unpolished rice ...	9.0	1.65	75.52	1.08	12.75
Polished rice ...	7.7	0.25	77.23	0.52	14.3
Parboiled rice ...	7.8	0.50	76.88	0.82	14.0
Malay rice ...	7.3	0.63	77.19	0.88	14.0

The average daily ration of an oriental labourer contains 1.3 lbs. of rice.

Dealing first with the differences in respect of proteins, that between parboiled and polished rices is small and within the limits of experimental error but admitting the difference to be a real one and assuming all the protein in rice to be available for purposes of nutrition, a person receiving parboiled rice would consume 9 grains more protein than one receiving polished rice and a person receiving Malay rice would consume 36 grains less than the one on polished rice. An explanation on this basis would therefore not be in accordance with the effects produced.

The amount of fat ingested in a ration of 1.3 lbs. of rice would be 45.5 grains in the case of parboiled rice, 22.75 grains in the case of polished rice and 57.25 grains in the case of Malay rice, differences which are appreciable and in accordance with the histological findings but which do not appear to furnish an adequate explanation if the fats be regarded merely as the esters of fatty acids.

The differences in respect of carbohydrates is small, parboiled rice contains more sugar than polished rice or Malay rice but it did not appear that these differences would furnish an explanation.

The ash furnishes a most imperfect idea of the salts contained in rice, all the organic salts being reduced to carbonates but there

is apparently a very constant difference in the amount of ash which is lower in the case of polished rice than in unpolished, parboiled or Malay rices. A person receiving parboiled rice would consume material yielding 74.6 grains of ash and a person receiving polished rice would consume material yielding 47.3 grains of ash. The difference of 27.3 grains is by no means inconsiderable more especially when it is remembered that small amounts of certain inorganic and organic salts have a great importance in the economy.

As we were not in a position to separate out the various salts occurring in rice and it seemed possible then that even if we did accomplish this the results might be unsatisfactory, it was decided to carry out investigations with a view to determining whether a poison or poisons existed in polished rices known to have been associated with outbreaks of beri-beri.

As has been already stated the white and the parboiled rices milled in this country have a common origin in respect of the grain from which they are prepared, therefore any deleterious substance or substances present in the white rice must have developed after polishing and have resulted from the action either of enzymes or of micro-organisms, failure to find these latter notwithstanding.

It has been suggested that the removal of the pericarp and subjacent layers deprives the grain of protective structures and facilitates the action of micro-organisms.

Rice is washed before cooking and the cooking involves exposure to a temperature of 212°F or thereabouts for some time, therefore the ingestion of living organisms with the rice may reasonably be excluded; their activity is confined to the interval which elapses between the milling and the cooking of the rice and the deleterious substance or substances produced by them must be capable of withstanding prolonged exposure to moist heat.

It might be suggested such harmful substances would be removed in the preliminary washing but the extent to which this washing is carried out varies greatly and, not infrequently, it is done in a perfunctory manner.

In the initial experiments for the isolation of a poison the white rice was macerated, or boiled and macerated, in water acidulated with a small quantity of Acetic Acid but on account of the rapidity with which moulds developed in the mixture and the difficulty of separating the fluid after maceration these experiments were discontinued.

In the next series of experiments alcohol acidulated with Acetic Acid was employed. The alcoholic extracts were concentrated *in vacuo* and finally freed from alcohol by exposure in evaporating basins to a moderate heat. The extracts were then examined and treated in every way possible for the isolation of poisons but although numerous examinations and experiments were made a poison or poisons were never isolated. The failure to obtain a positive result did not exclude a poison but when it is remembered that a large number of methods were employed it is suggestive that further work along these lines was unlikely to be profitable. We had already noted nutritive differences in the rices which in the light of our then knowledge we did not consider adequate to furnish the explanation sought for but failure to find a satisfactory result on a poison hypothesis caused us to turn our attention to further experiments on the nutritive hypothesis.

Failing chemical methods there remained animal experimentation and it was anticipated that by feeding various animals on rice and rice products, information of value might be obtained.

Feeding Experiments on Animals.

It was designed in the first instance to carry out experiments with anthropoids which we considered were more likely to yield information of value than experiments with animals lower in the zoological scale. Though anthropoids of several species are comparatively common in this country it was not without considerable difficulty that we were enabled to secure a sufficient number of them to make the experiment. Finally six were obtained. These were confined in separate cages and received cooked white rice and water. The results were unsatisfactory, as shortly after the beginning of the experiment several of the animals refused to eat the rice and all of them died of dysentery.

Six monkeys were fed in a similar manner. All of them became very much emaciated and five died after three months apparently of inanition. No degenerative changes were found in the nerves.

These experiments having therefore ended in failure recourse was had to experimentation with fowls, which previous observers had shown to be readily susceptible to a form of polyneuritis following the ingestion of certain foodstuffs.

Eijkman (12) was the first to observe among fowls kept at the Government laboratory at Weltevreden, Java, spontaneous polyneuritis characterised by degeneration of peripheral nerves and atrophy of the ganglion cells in the anterior cornua of the spinal cord. This condition he attributed to feeding with cooked rice. Experimentally he could produce the disease with decorticated, cooked, and raw rice but could prevent it by the addition of the fine inner capsules of the rice grain, the so-called silver layer (Zilvervliesjes) and could also cure it in the same way. By the administration of different forms of pure starch a similar disease was produced, potato starch was

the only one that proved to be harmless. Eijkman was of opinion that a toxic substance in rice and the other varieties of starch, developed in the crops of the fowls.

He adds that his investigations show that an apparently physiologically perfect diet can produce severe disease conditions and lead to death.

Eijkman repeated his experiments in Holland and found that the changes in the peripheral nerves were the most important features found on post-mortem examination. They concerned the sensory as well as the motor fibres. They involve bundles of the nerve trunks and present the picture of a non-inflammatory atrophic degeneration such as is seen when a nerve is cut off from its trophic centre. Certain changes in the posterior nerve roots also occur, these likewise show the characters of degeneration and atrophy. The muscles innervated by the affected nerves when treated with osmic acid show a large number of fine fat globules. Feeding with decorticated rice, raw or cooked, and immaterial of the origin or quality of the padi, produced the disease in three or four weeks.

In later investigations Eijkman fed fowls with ground rice and water rolled into balls, and the animals developed polyneuritis. Also in fowls which died after $3\frac{1}{2}$ months fed on husked barley he found many degenerated fibres in the sciatic nerve. If the fowls were fed on unhusked rice that had been heated in a steriliser at 115° or 125°C for two hours the animals developed polyneuritis, after 23 days in the case of rice heated to 115°C and after 21 days in the case of rice heated to 125°C. Simple boiling did not destroy the protective value of unhusked rice. Sterilised barley as well as rye and millet acted in the same way as rice.

Grijns (13) continuing the work of Eijkman found that by adding a certain quantity of Katjang idju (Phaseolus radiatus) to a diet of peeled rice the onset of polyneuritis in fowls was prevented.

Hulshoff Pol (14) pursuing the line of research suggested by the experiments of Grijns has shown that Katjang idju has prophylactic and remedial properties in beri-beri. He has further shown that a decoction from a moderately large quantity of Katjang idju possesses the same prohylactic and curative properties as the Katjang idju itself. Dr. Pol considers that an acid ("X" acid) separated from the decoction is the active principle but owing to difficulties in the way of the preparation of this substance no experiments have yet been made with it.

Kiewit de Jonge (15) has also tested the value of Katjang idju as a prophylactic and therapeutic remedy in beri-beri. He carried out an admirably planned and extensive investigation in the Lunatic Asylum at Buitenzorg and fully confirms the work of Grijns and Pol. The very valuable work of the Dutch School will be referred to later in conjunction with the results that have emerged from our own work.

In view of the results already attained by the Dutch physicians it was considered probable fowls would serve our purposes and a preliminary experiment was made by feeding fowls on white rice which was known to have been associated with an outbreak of beri-beri. A control group was fed on parboiled rice.

The fowls were confined in separate cages and were in all respects under identical conditions. The manner of arrangement of the cages is shown in Fig. I. The cages which were open at the bottom rested on a hard layer of clay the surface of which was covered with sand, and though the possibility of the fowls obtaining such things as worms and the like was not excluded, the conditions were more natural than if the cages had rested on a wooden or concrete floor.

In addition to rice which was supplied twice daily at 10 a.m. and 3 p.m. a small tin filled with water was placed in

I. General View of cages.

each compartment. The original weight of each fowl was noted and thereafter they were weighed once weekly at 12 noon.

A record of each experiment is given in the Tables which follow. The weights of the fowls in each successive week are furnished. The time of development of polyneuritis is indicated and whenever possible the analysis of the actual product supplied in the experiment is noted at the foot of the table.

In the group of fowls on white rice (Experiment No. 1) the first sign of disease was noted in fowl No. 1 on the 26th day of the experiment. In the morning it was observed that there was weakness of the leg muscles and consequent uncertainty of gait. The appearance of the fowl on this day is shown in Fig. II instead of standing upright it reclined on its side in the manner shown. When stimulated it would move about in an uncertain way. Examination of the blood failed to reveal the presence of any parasite or other abnormality.

Two days later the paralysis had advanced very considerably. It was quite unable to walk and the wing muscles were also involved so that these drooped. The diet was now changed to parboiled rice and padi but it was unable to eat and on the 9th day of the illness the fowl died. On post-mortem examination no macroscopic change was noted, there was no effusion into the serous cavities and no marked dilatation of the heart. The principal nerves of the legs and wings were preserved and on subsequent examination shewed characteristic Wallerian degeneration.

To test the possibility of the disease being a communicable one and unconnected with the diet, a fowl (No. 1 of the parboiled rice group) was transferred to the cage occupied by the fowl suffering from polyneuritis on the 5th day of the disease. This fowl was continued on parboiled rice as heretofore and remained healthy at the conclusion of the experiment five weeks later.

Fowl No. 4 developed the disease on the 28th day of the experiment. At 10 a.m. it was noticed to be unsteady but was able to walk about. Two hours later paralysis had extended greatly and the fowl rolled about in the cage in an endeavour to recover the upright position. The head was markedly retracted and when extended immediately returned to this position. The wings were drooping. An attempt was made to feed the fowl with padi but it was unable to eat owing apparently to the spasm of the neck muscles. On the following day the animal died.

This type of case, which was of the convulsive form of polyneuritis of fowls, was not infrequently observed throughout the experiments. It was less common however than the type in which simple paralysis was the principal feature.

On the day of onset of the disease a fowl was transferred from the parboiled rice group and placed in the cage to test the possibility of infection. The result in this as in numerous subsequent tests entirely negatived the possibility of the disease being communicable by contact or through intermediate hosts as ticks or lice.

Within eight weeks, eight of the twelve fowls had developed polyneuritis. Though the experiment was continued for four weeks longer, the other four fowls remained healthy.

The group of fowls on parboiled rice (Experiment No. 2) originally numbered eight. This number was afterwards increased as fowls from this group were transferred to cages occupied by fowls suffering from polyneuritis. In all twelve fowls were under observation for periods varying from five weeks to thirteen weeks. At the conclusion of the experiment all remained healthy. The weights of the fowls in grammes at various stages of the experiment are shown in Table No. 2.

II. Fowl fed on Siam Rice.
Polyneuritis. First day of disease.

Experiment No. 1.—White Rice (No. 2 Siam Depôt).

No	Description.	Original wt.	1st week	2nd week.	3rd week.	4th week	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.	11th week.	12th week
1	Grey hen	1220	1247	1294	1282	Polyneuritis								
2	Brown hen	634	630	607	565	505	498	503	525	590	642	637	627	607
3	Brown hen	848	862	870	807	760	Polyneuritis							
4	Red cock	1280	1335	1255	1132	996	Polyneuritis							
5	Black hen	735	690	668	640	572	572	Polyneuritis						
6	Red hen	840	900	878	854	703	Polyneuritis							
7	Black cock	637	637	577	560	507	492	455	460	Polyneuritis				
8	White hen	777	717	555	518	518	555	542	529	535	590	577	665	662
9	Brown hen	1210	1177	1107	1128	954	957	950	957	942	950	1015	910	905
10	Black cock	864	915	902	910	820	798	Polyneuritis						
11	Grey cock	822	835	787	715	718	701	688	687	722	740	707	725	715
12	Red cock	1655	1790	1735	1600	1475	1466	Polyneuritis						

Protein.	Fat.	Carbohydrate.	Ash.	Moisture.
7.80	0.15	77.49	0.56	14

$P_2 O_5$

0 28

Experiment No. 2.—Parboiled Rice.

No.	Description.	Original wt.	1st week.	2nd week	3rd week.	4th week	5th week.	6th week.	7th week	8th week.	9th week.	10th week.	11th week	12th week	13th week
1	Brownish hen	1295	1350	1355	1345	1435	1495	1465	1482	1548	1548				
2	White cock	1485	1565	1488	1275	1514	1512	1532	1568	1619	1624	1635	1645	1640	1662
3	Black hen	625	630	630	640	690	655	672	677	684	672				
4	Yellow hen	1115	1155	1222	1295	1417	1400	1407	1375	1305	1262				
5	Grey hen	700	715	717	702	702	702	724	727	725	752				
6	White hen	625	652	654	646	646	642	657	660	675	693				
7	Black cock	882	854	848	842	857	807	805	793	748	730				
8	Brown cock	865	857	848	804	840	827	842	834	835	855				
9	Yellow hen	1119	1120	1045	1048	1037	1052								
10	Black hen	870	887	877	866	875	877	882							
11	Brown hen	1039	1007	982	813	840	848	872							
12	Black hen	837	832	837	872	920	898	908	862	900	882	885			

It was thus shown that fowls were sensitive to differences between these two kinds of rice and the occurrence of polyneuritis furnished a reaction which has proved of the utmost value.

In this as in subsequent experiments every possible effort was made to transmit the disease to the fowls of the control groups but with uniformly negative results.

The previous researches had shown conclusively that white rice was either the vehicle by which the agent producing beri-beri was introduced into the body or that white rice was deficient in some substance or substances essential for metabolism. Parboiled rice neither conveyed this disease producing agent nor was it deficient in the substance or substances essential for metabolism.

By following up the results of these initial experiments on fowls we thought that it might be possible to determine the factors of importance in the etiology of beri-beri. In the case of poisons it should be possible to remove them from white rice and render it innocuous; if it were a deficiency it should be possible to supplement that deficiency either by the addition of the substances removed in the process of polishing or by the addition of substances extracted from parboiled or unpolished rice.

The Poison Hypothesis.

The chemical experiments referred to on page 27 had not been successful in isolating a poison recognizable by chemical means and the inoculation experiments performed on guinea-pigs, rabbits and monkeys with various substances isolated from white rice yielded no satisfactory result.

The disease which occurs among fowls fed on white rice provided a means of carrying out further experiments on the poison hypothesis. The existence of a poison or poisons in white rice had not at this time been definitely excluded and the further possibility remained that the harmful substance or substances could not be detected by any of the chemical reactions employed. Experiments were therefore undertaken with products extracted from white rices. In the first of these (Experiment No. 3) white rice obtained from the supplies issued during the inquiry at Durien Tipus was used. The rice was treated in the following manner:—

1. 1.5 kilogrammes of finely ground rice was macerated for four days in 1.5 litres of 94% alcohol.

2. The mass was then transferred to a percolator and percolated with the alcohol in which it had been macerated.

3. The mass was further percolated with 0.5 litre of 94% alcohol. This operation was repeated three times.

4. After percolation was complete the rice was removed, freed from alcohol by expression, and dried in the sun.

Five fowls were fed on the exhausted rice and three developed polyneuritis within five weeks.

It appeared therefore that percolation with cold alcohol had failed to dissolve out the hypothetical poison.

VI. Fowl fed on Siam Rice which had been extracted with 94 per cent. alcohol.
Polyneuritis. Severe case. Second day of disease.

EXPERIMENT No. 3.—White Rice after extraction with cold 94% alcohol.

No.	DESCRIPTION	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.
1	Brown cock	875	887	898	850	795	758	834	805	747	684	592
2	Grey hen	947	927	965	907	893	895	914	938	855	765	724
3	Black hen	732	722	712	680	597	Polyneuritis					
4	Red hen	895	879	845	804	742	Polyneuritis					
5	Black hen	785	788	750	640	Polyneuritis						

Protein.	Fat.	Carbohydrate.	Ash.	Moisture.
7·3	0.14	79.68	0.48	12.4

$P_2 O_5$

0.27

EXPERIMENT No. 4.—White Rice after extraction with hot 94% alcohol.

No	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week	9th week.	10th week.	11th week.
1	White cock	860	782	680	628	595	590	552	520	Polyneuritis	
2	Red hen	912	885	815	812	740	730	712	692	696	678
3	Yellow hen	713	653	555	515	516	492	470	445	429	427
4	Black hen	1275	1230	1107	1037	936	822	Polyneuritis			

Protein.	Fat.	Carbohydrate.	Ash.	Moisture.
7.0	0.11	79.01	0.48	13.4

$P_2 O_5$

0 27

EXPERIMENT No. 5.—Parboiled Rice + Extract from White Rice.

No	DESCRIPTION	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.	——
1	White cock	1532	1568	1619	1624	1635	1645	1640	1662	1650	In 51 days received concentrated extract from 5400 grammes W.R.
2	Black hen	872	920	898	908	862	900	860	882	885	Do. do.

In the second experiment (Experiment No. 4) rice from the same source was extracted with hot alcohol in the following manner:—

1. 1.5 kilogrammes of coarsely ground rice had poured over it 1.5 litres boiling 94% alcohol. It was allowed to macerate for several days.

2. The mass was then transferred to an apparatus for extraction with hot alcohol. This was an enlarged form of Soxhlet apparatus made of copper. The rice was treated in this apparatus by extraction with alcohol for 12 hours.

3. 300 c.c. alcohol were added on two occasions, the further extraction being continued for 8 hours.

4. The alcohol was expressed and the rice dried in the sun.

Four fowls were fed on the exhausted rice and two developed polyneuritis. Percolation with hot alcohol had also failed to dissolve out the hypothetical poison.

The bulk of the extracts from the rices had been employed in the chemical experiments but a quantity of the extract prepared by means of hot alcohol remained. This alcoholic extract had most of the alcohol removed from it by distillation *in vacuo* and was finally freed from alcohol by exposure in shallow basins at a low temperature. The alcohol-free extract was emulsified in distilled water and two fowls (Experiment No. 5) fed on parboiled rice received in addition daily an emulsion of the extract representing that obtained from 100 grammes of white rice. The experiment was continued for 51 days. Both fowls gained in weight and showed no signs of any disease.

These experiments showed that no alcohol soluble poison was contained in white rice.

In order to control the results of experiments with products obtained by extracting white rices with alcohol, parboiled rices were treated by precisely similar methods to those already described.

In the first of these experiments (Experiment No. 6) the product employed was obtained by treating parboiled rice with hot alcohol in an extraction apparatus of copper, the copper Soxhlet apparatus already described.

This proved to be an unsatisfactory method for the extraction of parboiled rice and the extraction apparatus shown in the figure was thereafter employed. The bottom of the thistle-head was covered with muslin, the ground rice and alcohol were heated for the requisite period, then by compression of the tube between the flask and the condenser it was attempted to force the fluid over the syphon which once filled would remove the menstruum completely. In practice, this was most troublesome and dangerous but by substituting a filter flask connected with a water-pump the difficulty was overcome and the subsequent extractions of rices were carried out with ease.

The technique employed in the extraction of rice by this method was as follows:—

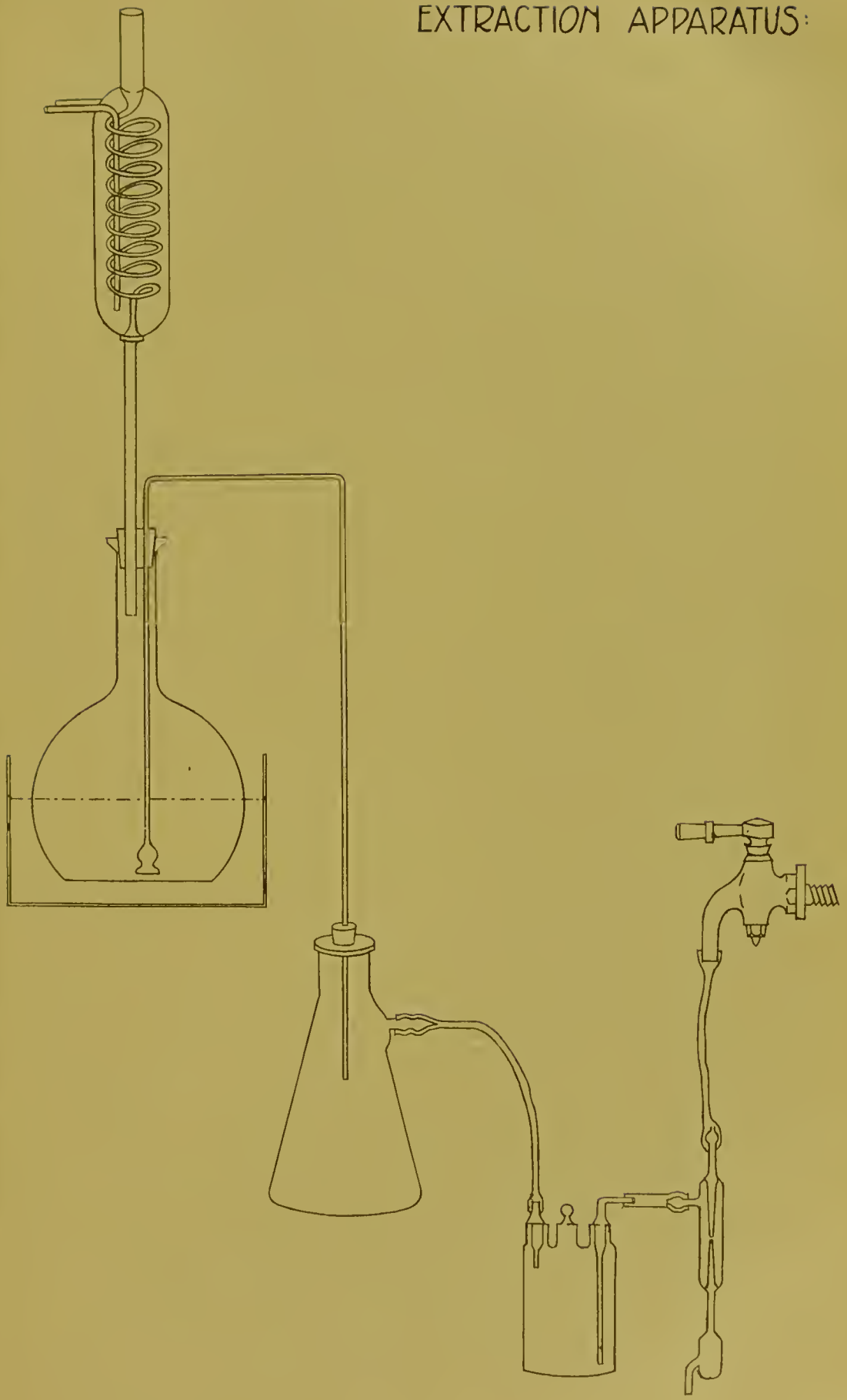
1. One kilogramme of rice was ground to a coarse powder and placed in a cylindrical jar. One litre of hot alcohol was poured over the rice and the mixture stirred daily for several days.

2. The mixture was transferred to a flask of 2250 cc. capacity and connected up with the extraction apparatus.

3. After heating for one hour the liquid was aspirated off.

4. Half a litre of alcohol was added to the partially exhausted rice, the mixture heated as before and the liquid aspirated off. This process was repeated four times.

EXTRACTION APPARATUS:



EXPERIMENT No. 6.—Parboiled Rice after extraction with hot 94% alcohol.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.
1	Yellow hen	567	560	517	450	425	412	450	448	437	442	475
2	Black cock	1295	1367	1260	1245	1150	1150	1139	1165	1125	1180	1195
3	Black cock	882	825	745	727	700	Polyneuritis					
4	Black hen	1150	1110	1012	947	892	925	979	962	965	925	967

Protein.	Fat.	Carbohydrate.	Ash.	Moisture.
7·9	0.08	80.82	0.6	10.5

EXPERIMENT No. 7.—Parboiled Rice after extraction with hot 94% alcohol.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week
1	Brown hen	1250	1245	1195	1035	890	920	
2	Yellow hen	1270	1290	1257	1255	1190	1120	
3	Black hen	1375	1340	1287	1160	1045	Polyneuritis	
4	Yellow cock	1615	1595	1550	1505	1295	1305	
5	Yellow hen	1257	1222	1115	1012	895	800	Polyneuritis
6	Black hen	1480	1485	1440	1350	1232	1095	Polyneuritis
7	White hen	1235	1040	930	835	740	655	
8	Brown hen	1450	1312	1200	1054	905	Polyneuritis	
9	Yellow hen	1245	1140	1080	1032	927	850	
10	Brown hen	1182	1154	1132	1172	1172	1127	
11	Yellow hen	1202	1075	1057	1015	990	985	
12	Black hen	1188	1140	1060	1005	930	845	

EXPERIMENT No. 8.—White Rice and extract from Parboiled Rice.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week	3rd week.	4th week.	5th week.
1	Brown hen	1365	1342	1235	1235	1287	1280
2	Red cock	1325	1180	1135	1143	1158	1155
3	Black cock	1325	1180	1170	1143	1137	1145
4	Brown cock	1285	1262	1258	1272	1252	1260
5	Red hen	1185	1215	1152	1112	1095	1062
6	Black cock	1355	1305	1372	1360	1362	1330
7	Brown hen	1325	1192	1205	1185	1167	1175
8	Grey hen	1225	1272	1295	1290	1310	1295
9	Brown cock	1650	1750	1760	1780	1772	1745
10	Black hen	1350	1348	1325	1315	1265	1290
11	Black hen	1343	1307	1300	1305	1352	1315
12	Brown hen	1240	1222	1205	1227	1262	1302

The exhausted rice was freed from alcohol by exposure to the sun.

The alcoholic extracts by whichever apparatus obtained, contained a quantity of insoluble matter, due to the coarseness of the method by which separation of the menstruum from the marc was effected. No attempt was made to separate the soluble and insoluble substances from these extracts by further filtration, and it is possible that the presence of insoluble substances in the liquid may explain the equivocal results obtained in the extraction of parboiled rice with 94% alcohol.

In Experiment No. 6 parboiled rice which had been ground and afterwards exhausted several times with hot 94% alocohol was used. It was anticipated that by this method such substances as lecithin and a portion of other fats would be removed from it. Four fowls only were employed for the experiment. In the fifth week one of them, No. 3, developed a condition indistinguishable clinically from other cases of polyneuritis in fowls fed on white polished rice.

This result indicated that some protective substance ordinarily present in parboiled rice had been removed by treatment with hot 94% alcohol and further experiments were undertaken to test the value of this suggestion.

In the experiments next to be described alcohol of 94% strength was employed. For the products employed in Experiments Nos. 7 and 8 an apparatus similar to that illustrated by the figure was employed. Here, however, a positive pressure generated by the boiling fluids within the flask was employed for withdrawing the liquid. The compact character of the rice mass made it difficult to secure more than very coarse filtration of the solution. Of twelve fowls fed on the exhausted rice in Experiment No. 7 two developed polyneuritis within five weeks and two more in the sixth week. Fowls fed on white rice to

which was added the extract from 83 grammes of unpolished parboiled rice daily (Experiment No. 8) all remained healthy.

It was concluded from these experiments that the protective substances were soluble in hot alcohol of 94% strength.

A further experiment, No. 9, was carried out with unpolished parboiled rice extracted with 94 % alcohol in the apparatus illustrated. One fowl died in the seventh week. No cases of polyneuritis occurred.

In the control experiment, No. 10, in which the extracted substances were added to a white rice diet, all the fowls remained healthy.

These latter results can only be explained by assuming that the essential substances were only partially extracted from the unpolished parboiled rice.

The next experiment, No. 11, was one in which parboiled rice from the same sample as used in Experiment No. 6 was treated with cold alcohol of 94% strength by maceration and percolation. At first five fowls only were employed in this group but as cases of polyneuritis occurred in fowls fed on white rice treated by identical methods, fowls were transferred to the latter group and continued on the same food to test the possibility of conveying infection. To replace these, fowls were added and in the course of the experiment eight fowls in all were under observation.

From an examination of the chart it will appear that while some of the fowls lost weight the general result was a gain in weight and all remained healthy at the conclusion of the experiment.

It would appear that cold 94% alcohol is not an effective solvent of the protective substances.

EXPERIMENT No. 9.—Parboiled Rice after extraction with 94% alcohol.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	
1	Black hen	815	772	795	780	795	762	815	810	
2	Brown hen	895	832	820	820	775	745	755	745	
3	Yellow hen	1070	1100	1035	955	975	960	1015	1005	
4	Brown hen	975	952	940	865	845	825	850	820	
5	Yellow hen	1100	1115	1050	1005	1020	937	950	935	
6	Yellow hen	960	960	920	875	860	845	855	850	
7	Red cock	1075	1010	1045	1000	975	880	815	735	
8	White hen	820	810	810	800	800	775	820	810	
9	White hen	890	912	920	870	840	790	770	720	
10	Yellow hen	1075	1080	1045	1050	1055	982	985	955	
11	Brown hen	990	1000	1010	1015	970	930	925	905	
12	Brown hen	1290	1110	1125	1060	955	915	815	Died.	No degeneration of nerves.

Protein	Fat.	Carbohydrate.	Ash.	Moisture.
7.60	0.06	80.66	0.88	10.80

$P_2 O_5$
0.425

Experiment No. 10.—White Rice + Extract from Parboiled Rice.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.
1	Yellow hen	1092	1150	1120	1020	1010	1000	1000	970
2	Brown hen	1065	1052	1010	935	905	900	905	910
3	White hen	1272	1185	1200	1180	1170	1215	1200	1210
4	Brown hen	1048	1085	1085	1005	920	955	975	960
5	White hen	1315	1295	1230	1220	1232	1220	1255	1245
6	Brown hen	1340	1260	1275	1280	1277	1265	1290	1220
7	Brown cock	1415	1390	1425	1550	1590	1590	1570	1385
8	Red cock	1495	1085	Died	Cause of death not determined.				
9	White hen	1450	1480	1425	1285	1245	1255	1265	1220
10	Grey hen	1260	1130	1175	1215	1280	1275	1280	1215

	Protein.	Fat.	Carbohydrate.	Ash.	Moisture.
White Rice	6.90	.20	77.26	0.40	15.24

$P_2 O_5$

0.22

EXPERIMENT No. 11.—Parboiled Rice after extraction with cold 94% alcohol.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.
1	Black hen	852	827	815	770	735	715	687	648	552	475	445
2	Red hen	875	910	930	1007	1019	1037	1012	1002	988	965	940
3	Red hen	804	820	837	822	837	835	867	852	870	Transferred.	
4	Yellow cock	1097	1137	1208	1210	1287	1298	1315	1325	1345	1402	
5	Black hen	625	640	609	595	577	595	572	598	605	655	
6	Yellow hen	900	889	844	846	798	763	705				
7	Red cock	1208	1184	1230	1188	1165	1074	1032				
8	Red hen	874	875	874	895	890	882	850				

Protein.	Fat.	Carbohydrate.	Ash.	Moisture.
8.0	0.06	78.0	0.78	12.7

EXPERIMENT No. 12.—Parboiled Rice after extraction with hot Proof Spirit.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week
1	Black cock	1490	1350	1250	1150	1030	935	
2	Black hen	1490	1475	1340	1300	1240	1190	
3	Brown cock	1275	1235	1140	1110	1030	Polyneuritis	
4	Yellow hen	1210	1245	1165	1095	1005	925	
5	Yellow hen	1130	980	935	835	740	665	
6	Red cock	1390	1355	1250	1080	Polyneuritis		
7	White hen	1435	1370	1280	1190	1160	1060	
8	White cock	1465	1325	1180	1080	995	910	
9	Yellow hen	1335	1217	1080	985	855	890	
10	Black cock	1560	1420	1325	1155	Polyneuritis		
11	Yellow hen	1390	1325	1255	1190	1130	1095	
12	White cock	1475	1285	1170	1080	985	Polyneuritis	

Protein.	Fat.	Carbohydrate.	Ash.	Moisture.
7.65	0.10	80.01	0.44	11.8

$P_2 O_5$

0.29

Experiment No. 13.

White Rice + Proof Spirit extract from Parboiled Rice.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Red cock	1195	1160	1180	1215	1175	1182
2	Red cock	1320	1312	1310	1310	1310	1292
3	Red cock	1122	1060	1050	1052	1050	1030
4	Yellow cock	1315	1320	1310	1385	1410	1390
5	Red cock	1420	1390	1425	1395	1370	1342
6	Yellow cock	1700	1710	1715	1740	1725	1722
7	White hen	1820	1690	1660	1710	1780	1740
8	Grey hen	1870	1910	1935	1760	1840	1930
9	Red cock	1710	1705	1687	1740	1765	1770
10	Yellow cock	1640	1650	1650	1660	1660	1655

In Experiments No. 12 and 13 unpolished parboiled rice was extracted with alcohol of proof spirit strength in the apparatus figured. The extracts freed from alcohol in the manner already described were used in these experiments.

In Experiment No. 12 twelve fowls were fed on the exhausted rice; three developed polyneuritis within five weeks. In the corresponding experiment, No. 13, in which the alcohol-free extract obtained from 60 grammes of unpolished parboiled rice was added daily to the diet of twelve fowls on white polished rice, all the fowls remained healthy for five weeks.

This result shows that the essential protective substances are also soluble in alcohol of proof spirit strength.

In the foregoing series of experiments, which were of a tentative nature, no evidence was obtained that white (polished) rice ever contained a beri-beri producing agent. The results taken in conjunction with the histological findings suggested that by the removal of the subpericarpal layers of the grain in the process of milling, the grain is deprived of some substance or substances of high physiological value.

In subsequent experiments this conclusion was abundantly confirmed. In giving a detailed record of these experiments therefore, these are arranged in accordance with the conclusions ultimately arrived at and not in chronological order. It is hoped that in this way the position will be made clearer.

Experiments with various White Rices.

Experiments were carried out with white polished rices from various sources. These may properly be grouped in a single series.

In the first experiment of the series (Experiment No. 14) the white polished rice used in the inquiry at Durian Tipus was employed. Of the four fowls used for the experiment three developed polyneuritis within four weeks.

The second experiment of the series (Experiment No. 15) was one designed to test the effect of feeding fowls on a white polished rice while at the same time another group (Experiment No. 25) was fed on the original partially husked padi from which this white rice had been prepared, and yet another (Experiment No. 32) on the white polished rice to which the substances removed in the polishing process had been added. In the group under review, those to which white rice alone was given, one fowl (No. 8) died on the third week without signs of polyneuritis—six others developed polyneuritis within five weeks.

The third experiment of the series (Experiment No. 16) was carried out to test whether, as alleged by Braddon, rice that had become stale on account of changes occurring in it in the interval between milling and consumption was more harmful in its influence than freshly milled rice. An assistant was stationed in Singapore who forwarded to the laboratory daily by the most expeditious route a quantity of white polished rice milled on the day of despatch. Of twelve fowls fed on this rice five developed typical polyneuritis within four weeks, a result similar to that in experiments in which white polished rice varying in age from a few months to two years was employed.

III. Fowl fed on Siam Rice.
Polyneuritis. Mild case. Eighth day of disease.

IV. Fowl fed on Siam Rice.
Polyneuritis. Mild case. Fifth day of disease.

EXPERIMENT No. 14.—White Rice (from Beri-beri outbreak Durien Tipus).

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.
1	Black cock	2098	2144	1892	1915	Polyneuritis		
2	White hen	1292	1222	1090	960	817	667	672
3	Yellow cock	1439	1300	1080	969	Polyneuritis		
4	Brown hen	1090	1020	952	880	Polyneuritis		

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
7.45	0.17	78.02	13.85	0.51

$P_2 O_5$

0.28

Experiment 15.—White Rice (No. 2 Siam milled in Singapore).

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Yellow hen	1052	1035	1050	1072	1090	1060
2	White hen	1215	1265	1187	Polyneuritis		
3	Red cock	1555	1452	1295	1115	Polyneuritis	
4	Black hen	1300	1240	1115	1012	880	Polyneuritis
5	Brown hen	882	807	800	770	785	755
6	Black hen	872	830	905	905	862	820
7	Black cock	855	802	785	692	Polyneuritis	
8	Brown cock	730	650	545	Died.	No Polyneuritis	
9	Yellow hen	1200	1110	985	865	768	Polyneuritis
10	White hen	1440	1465	1248	1075	982	Polyneuritis
11	White hen	1470	1415	1380	1192	1095	1030
12	White hen	1178	1185	1032	945	860	875

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
7·3	0.40	77·43	14.3	0.57

$P_2 O_5$

0.28

Experiment No. 16.—White Rice (recently milled).

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Red cock	1570	1500	1320	1255	Polyneuritis	
2	White hen	810	830	795	757	725	
3	Brownish hen ..	1327	1138	1048	935	725	
4	Black hen	815	815	732	717	Polyneuritis	
5	Brown hen	1250	1207	1088	955	Polyneuritis	
6	Red cock	1135	1102	982	Polyneuritis		
7	White hen	895	875	777	657	685	
8	Yellow cock	1100	1020	960	892	855	
9	Grey hen	1173	1175	1080	935	815	
10	Black cock	1225	1203	1098	930	Polyneuritis	
11	White cock	1010	890	762	632	545	
12	Yellow hen	892	822	750	684	591	

Protein.	Fat.	Carbohydrate.	Ash.	Moisture.
8.1	0.41	76.76	0.47	14.26

$P_2 O_5$
0.27

Experiment No. 17.—White Rice (No. 1 Siam).

No	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.
1	Red cock	1385	1370	1342	1382	1365	Polyneuritis				
2	Brown hen	1045	1075	1060	1107	1050	965	895	945	980	1015
3	Yellow cock .. .	1040	1027	987	1060	1032	Polyneuritis				
4	Grey hen	1095	1115	1045	1032	965	930	905	940	965	Polyneuritis
5	Red hen	1070	1052	1045	975	930	907	935	950	975	990
6	Yellow hen	1435	1418	1305	1222	1177	Polyneuritis				
7	Yellow hen	1230	1180	1102	1037	990	940	940	900	880	920
8	Brown hen	1645	1625	1505	1465	1390	1257	1215	Polyneuritis		
9	Yellow cock	2035	2080	1900	1845	Polyneuritis					
10	Red cock	1550	1635	1497	1480	1435	Polyneuritis				
11	White cock	1425	1440	1337	1335	Polyneuritis					
12	Red cock	1550	1608	1495	1440	Polyneuritis					

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
6.9	0.20	77.26	15.24	0.40

$P_2 O_5$

0.22

Experiment No. 18.—White Rice (No. 1 Siam).

No.	Description.	Original wt.	1st week.	2nd week.	3rd week	4th week.	5th week.	6th week
1	Brown hen	1290	1185	1175	1160	1182	Polyneuritis	
2	Red cock	1190	1160	1150	1125	Polyneuritis		
3	Black cock	1140	1180	1145	1145	Polyneuritis		
4	Brown cock	1275	1210	1145	Polyneuritis			
5	Red hen	1045	940	892	770	Polyneuritis		
6	Black cock	1310	1270	1220	1222	Polyneuritis		
7	Brown hen	1185	1195	1180	1060	955	Polyneuritis	
8	Grey hen	1255	1155	1055	990	990	1035	1050
9	Brown cock	1820	1780	1720	1690	Polyneuritis		
10	Black hen	1210	1170	1132	992	Polyneuritis		
11	Black hen	1300	1290	1262	1152	Polyneuritis		
12	Brown hen	1410	1415	1355	1332	1282	Polyneuritis	

Experiment No. 19.

White Rice (washed thoroughly before feeding).

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	Brown cock	1575	1455	1350	1330	Polyneuritis
2	White hen	1430	1350	1180	1060	
3	Black hen	1385	1315	1190	1150	
4	Red cock	1505	1450	1280	Polyneuritis	
5	White hen	1575	1430	1310	1220	
6	Brown hen	1325	1270	1090	1015	
7	Black hen	1390	1310	1130	1020	
8	Red cock	1930	1790	1485	Polyneuritis	
9	Brown hen	1100	1055	965	895	
10	Black hen	1100	1035	980	975	
11	White hen	1530	1415	1360	1205	
12	Brown cock	1415	1340	1235	1180	

EXPERIMENT No. 20.—White Rice (No. 1 Siam).

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	Brown cock	1135	1155	1160	1055	Polyneuritis
2	Yellow cock	1280	1230	1165	1010	Polyneuritis
3	Black cock	1180	1200	1225	1155	Polyneuritis
4	Brown cock	1150	1145	1170	1105	1050
5	Black cock	1175	1200	1100	985	Polyneuritis
6	Yellow cock	1090	990	955	910	780

Wallerian degeneration.
Teased preparation from Sciatic nerve of fowl suffering from Polyneuritis.

Experiments Nos. 17 and 18 were carried out with white polished rice of the best quality (No. 1 Siam) purchased locally. In one of them nine fowls out of twelve developed polyneuritis within nine weeks, seven cases occurring before the end of the fifth week. In the other, eleven cases among twelve fowls occurred within five weeks. It appeared in these experiments that certain fowls were more resistant than others to the injurious influence of white rice. The reason for this was not ascertained though the observation was made that such fowls were commonly the younger members of the group, while those who succumbed most quickly were the full grown fowls who consumed a large quantity of rice. It was also noted that after two or three weeks fowls on white rice usually showed a disinclination to eat.

These observations which are similar to those made when human beings were under observation appear to us to be irreconcilable with the idea of simple deprivation of proteins, fats or carbohydrates being the final explanation of the occurrence of the disease.

Experiment No. 19 was carried out with a white polished rice as a control to other experiments in which this same sample was employed as the principal article of diet, various materials being added to it. Experiment No. 20 was carried out for a similar purpose.

In every case of polyneuritis Wallerian degeneration (Photo) was demonstrated microscopically in the affected nerves. The changes are apparently identical in every way with those found in the peripheral nerves in beri-beri. An extensive investigation of all the materials obtained is now being carried out by Dr. Fletcher.

Rangoon Rice.

There is evidence that the variety of white polished rice known as Rangoon rice is less harmful than Siam rice. Braddon

quotes examples in support of this view and instances have fallen under our own observation.

In Experiment No. 21 Rangoon rice was employed. Fowls No. 10 and No. 12 died in the 5th and 4th week respectively but there was no clinical or pathological evidence that they suffered from polyneuritis. Both had a form of purulent conjunctivitis.

No. 4 (Fig. 5) was a typical case of polyneuritis in the 5th week and no other case occurred until the 15th week when Nos. 7 and 8 suffered from it. All three cases resulted fatally and the diagnosis was confirmed post-mortem.

Such evidence as is furnished by this single experiment confirms the experience of those who assert the comparative harmlessness of Rangoon rice.

The explanation of this observation is a matter for further inquiry. Whether the padi grown in Burmah is richer in protective substances and therefore can afford to lose in the polishing process a greater quantity of them without harmful result or whether there is some important difference in the milling process it is impossible yet to say. From histological examination of the grains we incline to the former view.

V. Fowl fed on Rangoon Rice.
Polyneuritis. Severe case. First day of disease.

Experiment No. 21.—White Rice (Rangoon).

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.	11th week.	12th week.	13th week.	14th week.	15th week.
1	Red cock	1104	1147	1132	1175	1174	1197	1192	1237	1185	1210	1190	1195	1202	1248	1185	1205
2	White hen	857	888	854	810	830	822	818	765	799	788	762	760	715	658	545	Died
3	Black hen	945	940	905	857	788	757	735	700	682	630	554	480	Died			
4	Yellow hen	1227	1207	1150	995	850	Polyneuritis										
5	Black cock	1280	1285	1327	1262	1293	1307	1352	1248	1282	1280	1250	1240	1252	1255	1215	1155
6	Black hen	1225	1252	1252	1265	1322	1425	1492	1471	1529	1395	1398	1440	1422	1352	1245	1170
7	Red cock	957	955	910	885	952	922	898	829	787	730	705	660	655	632	607	Polyneuritis
8	White cock	745	769	765	662	625	639	622	602	565	539	535	530	512	485	450	Polyneuritis
9	Grey hen	667	672	667	675	693	707	700	675	712	677	691	737	734	747	740	735
10	Black cock	729	757	657	548	465	Died										
11	Grey hen	637	637	632	595	614	552	489	439	467	479	460	445	430	422	409	395
12	Black cock	845	852	747	595	Died											
13	Red cock	1239	1127	1140	1180	1177	1185	1205	1210	1220	1185	1120	Replaced No. 4.				
14	Brown hen	1032	1045	1077	1087	1154	1160	1215	1230	1245	1252	1190	1100	Replaced No. 10.			
15	Brown hen	922	952	983	970	975	1004	998	1000	995	965	942	860	Replaced No. 12.			

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
7.3	0.63	77 15	14.18	0 74

$P_2 O_5$
0.33

Experiment No. 22.—Simple Starvation.

No	Description.	Original wt	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Brown hen	1170	1052	960	868	842	
2	Yellow hen	1365	1230	1115	1070	1024	
3	Black hen	1085	940	859	782	750	
4	Yellow cock	1490	1335	1225	1122	1070	

Experiment No. 23.—Simple Starvation.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	——
1	Yellow hen	1612	1500	1355	1275	1200		
2	Yellow cock	1285	1170	1030	930	815		Killed for examination of nerves 5th week. No degeneration.
3	Yellow hen	1860	1790	1615	1490	1400		
4	Red cock	1470	1385	1190	925			Killed for examination of nerves 4th week No degeneration.
5	Brown hen	1010	915	835	760	675		
6	Red cock	1420	1325	1120	930	720		Died 5th week. No degeneration of nerves.
7	Yellow hen	1350	1270	1220	1100	1000		
8	Red cock	1720	1640	1455	1350	1245		
9	Black hen	1850	1765	1615	1500	1395		
10	Red cock	1360	1280	1150	1035	950		
11	Red cock	1430	1345	1090	880			Died 4th week. No degeneration of nerves.
12	Yellow hen	1510	1400	1237	1155	1100		

Starvation and Forced Feeding Experiments.

As has been already stated it was usually found that after the first few weeks on a white rice diet, most fowls showed a disinclination to eat. Commonly also fowls lost greatly in weight before the development of polyneuritis. That these were by no means constant features however will be seen on reference to the tables which show that in some cases fowls continued to eat well and maintain their normal weight until the disease declared itself.

Despite the fact that groups of fowls on a diet of padi unpolished rice or parboiled rice usually continued to eat well throughout, the suggestion was raised that on account of the monotonous nature of a diet of white rice the fowls ceased to eat and that polyneuritis was a result of partial simple starvation. This was at all events a point that required to be tested and accordingly experiments were carried out.

In the first experiment of this series (Experiment No. 22) four fowls hitherto feeding on padi were employed. Nothing but water was supplied. The bottom of the cage being open they had access to such food, insects and the like, as could be obtained there—this condition of affairs was, however, controlled by the fact that all fowls including those on white rice were similarly circumstanced. Within four weeks all the fowls had lost greatly in weight and were weak but none of the signs constantly associated with polyneuritis had shown themselves. Within a similar period some fowls of a group fed on white polished rice had invariably shown signs of polyneuritis.

The number of fowls in the first experiment was of course too small to furnish any definite information and a second experiment with 12 fowls was begun (Experiment No. 23).

Within five weeks two of the fowls composing this group had died and two others were killed when their condition was such that it was apparent they would die within a few hours. None had showed any clinical signs of polyneuritis and careful examination of the nerves showed no characteristic degeneration changes.

A third experiment, No. 24, was carried out with a similar result.

It was concluded that polyneuritis of fowls is not due to general deprivation of food but to deprivation of some particular element in a diet of white polished rice.

In order to test further the correctness of this conclusion Dr. Fletcher carried out an experiment in which two groups of fowls were fed on white rice (Experiment No. 25).

To the first group (Fowls Nos. 1—6) in the following table 30 grammes of white rice was supplied twice daily in the ordinary way. To the second group Nos. 7—12, 30 grammes of white rice was passed into the crop twice daily.

The result showed that there was no difference between the two groups as to their liability to develop polyneuritis. Polyneuritis therefore cannot be due to simple deprivation of food.

An interesting observation made by Dr. Fletcher in connexion with this experiment was that when a fowl which was being forcibly fed suffered from the disease the normal digestive powers were markedly interfered with and the crop remained constantly distended with rice. If now a small quantity of polishings was passed into the crop the accumulated rice seemed to be dissolved and normal digestive power was speedily regained.

Experiment No. 24.—Simple Starvation.

No	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	——
1	Black hen	915	755	645	525	Died.	
2	Brown hen	1630	1370	1170	955	Died.	
3	Black hen	1225	1070	980	845	708	
4	Brown hen	1125	970	815	715	545	
5	White cock	1775	1530	1380	1295	1130	Killed in 5th week. Normal except globulation of few nerve fibres.
6	Brown hen	1000	830	740	660	575	
7	Brown hen	1505	1325	1210	1115	1020	
8	Brown cock	1440	1210	1060	925	655	
9	Yellow hen	1155	970	870	765	550	
10	Yellow hen	1160	990	895	825	755	

EXPERIMENT NO. 25.—White Rice. Nos. 1—6 Ordinary Feeding. Nos. 7—12 Forced Feeding.

No	DESCRIPTION	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	Red and black cock ..	1405	1320	1200	Polyneuritis	
2	White and black cock ..	1255	1190	1070	Polyneuritis	
3	Spotted cock	1620	1772	1680	1540	
4	Yellow and white cock ..	1385	1385	1250	1180	
5	Red and black cock ..	1285	1260	1050	730	Polyneuritis
6	Red and black cock ..	1340	1205	935	Polyneuritis	
7	White and brown cock ..	1000	1000	950	Polyneuritis	
8	Red and black cock ..	1635	1630	1570	Polyneuritis	
9	Black and red cock ..	1485	1440	1370	Polyneuritis	
10	Red cock	1485	1315	1200	Polyneuritis	
11	Yellow and white cock ..	1580	1580	1580	Polyneuritis	
12	Black and yellow cock ..	1630	1620	1370	Died Polyneuritis	

Experiment No. 26.—"Cargo" Rice.

No	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	——
1	Brown and yellow hen ..	910	862	848	857	900	952	940	Remains healthy
2	Brown and black cock ..	2005	2020	2085	2080	2062	2053	2052	,, ,,
3	Brown and red cock ..	1805	1840	1885	1920	1965	1982	1982	,, ,,
4	Black hen	1437	1450	1390	1290	1337	1235	1285	Injured 3rd week
5	Yellow hen	887	868	745	835	980	1022	1080	Remains healthy.
6	Brown cock . ..	1195	1265	1297	1325	1352	1375	1335	,, ,,
7	Brown hen	1205	1290	1405	1465	1530	1565	1425	,, ,,
8	Brown cock	1035	1075	1112	1158	1164	1185	1195	,, ,,
9	Brown hen	1012	1120	1130	1245	1265	1235	1205	,, ,,
10	White hen	805	857	840	820	798	785	775	,, ,,
11	Black hen	1048	1075	1097	1132	1152	1185	1190	,, ,,
12	Brown hen	978	1010	1018	1045	1103	1135	1210	,, ,,

Experiment with Unpolished Rices.

During the course of the experiments it was observed that fowls always remained in health on padi and fowls suffering from polyneuritis almost invariably recovered when given padi. The present experiment was one in which a partially husked rice known commercially as "cargo rice" was employed—about half the grains are denuded of husk. This experiment was carried out as a preliminary to certain experiments in which this same product, after milling in various ways, was employed These latter experiments will be referred to later.

In the experiment now under review, Experiment No. 26, twelve fowls were fed on partially husked rice for six weeks and all remained in good health at the conclusion of the experiment.

Other experiments in which padi and unpolished rices were employed are detailed elsewhere—all these experiments confirmed the observation that fowls remained in health when fed on rice which still retained its subpericarpal layers.

Experiments with Parboiled Rice.

Experiments with parboiled rice were carried out simultaneously with and for longer periods than experiments with white polished rice, as controls for the results obtained in the latter groups.

The experiment first described, No. 27, was one undertaken at the beginning of the series. In it five fowls only were employed. All maintained health and weight during fifteen weeks, cooked unpolished parboiled rice only being supplied throughout. It was thus shown that, in the conditions under which our experiments were carried out, a diet of parboiled rice

and water was sufficient to maintain fowls in health and weight over prolonged periods and this result repeatedly obtained was regarded as an adequate control to results obtained in shorter periods, five weeks or less.

In Experiment No. 28 that form of parboiled rice known as "muthu samba" which is prepared in India was employed. It is an expensive variety and is eaten only by the more wealthy members of the Indian community. Many fowls in this group showed a moderate gain in weight at the end of six weeks, a few showed a slight loss, all remained healthy throughout.

The next experiment of this series, No. 29, was used as a control to the results obtained in another group of fowls fed on on the same sample of rice after exhaustion with proof spirit (in the latter group four cases of polyneuritis occurred within six weeks). In the experiment now under discussion, which was continued for ten weeks, though most of the fowls lost materially in weight and one died in the 9th week, none showed recognisable signs of polyneuritis.

In Experiment No. 30 parboiled rice in a finely ground state was used as a control to the results in groups in which a similar product after exhaustion with alcohol was employed.

It is apparent from an analysis of the results in this series of experiments that marked differences exist in the nutritive value of different samples of parboiled rice. These differences are due to two factors, the initial richness of the grain and the extent to which the polishing process has been carried out. It does not appear that the method of treatment by parboiling before husking operates in any way other than to harden the external layers of the grain rendering them less easy of removal.

Experiment No. 27.—Unpolished Parboiled Rice.

No	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.	11th week.	12th week.	13th week.	14th week.	15th week.
1	Black hen	822	755	740	740	700	700	720	700	710	710	715	704	725	727	747	760
2	Red hen	727	785	770	770	740	740	730	740	740	750	745	765	785	790	825	850
3	Black hen	642	665	645	650	630	630	620	630	635	630	625	635	635	657	700	700
4	Grey hen	807	810	807	810	790	820	820	830	830	850	870	850	860	864	902	912
5	Brown cock	825	875	872	855	810	790	810	810	800	830	802	789	785	792	820	860

Experiment No. 28.—Parboiled Rice (Indian).

No.	Description	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.
1	Brown hen	1300	1300	1298	1292	1290	1270	1270
2	Red cock	1180	1182	1194	1185	1160	1175	1190
3	Black cock	1192	1155	1152	1130	1125	1140	1140
4	Brown cock	1292	1322	1320	1277	1280	1250	1275
5	Red hen	1117	1130	1094	1075	1005	1025	1045
6	Black cock	1345	1345	1339	1307	1305	1325	1310
7	Brown hen	1212	1192	1185	1165	1205	1210	1185
8	Grey hen	1210	1190	1167	1187	1245	1215	1255
9	Brown cock	1660	1810	1850	1852	1835	1790	1820
10	Black hen	1280	1340	1307	1245	1200	1190	1210
11	Black hen	1272	1335	1295	1285	1280	1275	1300
12	Brown hen	1350	1395	1425	1484	1550	1545	1410

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
6.3	0.2	78.31	14.35	0.84

$P_2 O_5$

0.345

Experiment No. 29.—Parboiled Rice (Penang).

No.	Description	Original wt.	1st week.	2nd week.	3rd week.	4th week	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.
1	Yellow hen	1315	1340	1310	1200	1155	1125	1100	1065	1040	1005	972
2	White cock	1300	1285	1240	1205	1155	1135	1120	1050	1005	1050	1045
3	Yellow hen	920	900	905	915	839	820	810	802	840	790	775
4	Red cock	1390	1435	1450	1470	1435	1380	1405	1420	1445	1460	1398
5	Yellow hen	1270	1220	1140	1055	1005	1005	955	885	825	760	695
6	Black cock	1380	1385	1370	1325	1310	1310	1295	1272	1275	1255	1235
7	Yellow hen	1345	1370	1385	1380	1295	1270	1240	1082	955	Died	No Polyneuritis
8	Yellow cock	1700	1700	1565	1565	1547	1545	1505	1515	1500	1470	1505
9	Brown hen	1450	1355	1350	1335	1220	1205	1200	1109	1098	1010	885
10	Yellow cock	1475	1465	1410	1385	1420	1410	1355	1395	1400	1395	1380
11	Yellow hen	1195	1205	1190	1130	1122	1090	1160	1084	1040	1060	1030
12	Black cock	1590	1600	1585	1540	1510	1470	1480	1472	1458	1445	1455

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
7.80	0 50	76.62	14.30	0.78

$P_2 O_5$
0.41

EXPERIMENT No. 30.—Parboiled Rice (Penang) Finely Ground.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.
1	Black hen	1210	1225	1180	1285	1280	1245	1240
2	White hen	1320	1275	1239	1265	1255	1272	1260
3	Grey hen	1450	1430	1337	1385	1405	1352	1360
4	Brownish hen ..	1235	1205	1187	1172	1140	1150	1155
5	Brown cock	1185	1195	1157	1170	1150	1079	1028
6	Brown hen	1225	1240	1246	1277	1280	1220	1215
7	White hen	1305	1280	1238	1257	1265	1275	1227
8	Black hen	1185	1190	1191	1245	1237	1202	1135
9	Brown hen	1240	1165	1055	1072	1102	1100	1090
10	Black hen	1330	1290	1173	1195	1177	1170	1145
11	White hen	1305	1295	1219	1280	1235	1195	1152
12	Brown cock	1375	1335	1304	1300	1250	1222	1172

Experiments with Malay Rice.

It is conceded by all those whose knowledge of this country and of its people enable them to speak with authority upon the point that among Malays under primitive conditions beri-beri is very rare.

Braddon states that "among these natives so long as they lead their primitive pastoral and agricultural life untouched by the influences which march with a civilization represented by encroaching hordes of Chinese beri-beri never occurs."

Hamilton Wright (16) says "My own experience of Malay Kampongs (Malay villages) is that beri-beri is almost unknown in them. The farther the Malay population is removed from centres of civilization the less beri-beri is seen in it."

Daniels (17) says "Malays living in Kampongs are the only class that do not suffer from beri-beri."

An account has already been given of the Malay method of preparing rice.

The first experiment in this series (Experiment No. 31) was one in which a rice prepared from a locally grown padi was employed. This was obtained from the Kuala Pilah district through the kindness of the District Officer, Mr. Eric Dickson, and Dr. Lucy.

A Malay woman prepared rice from this padi after the manner and with the primitive implements used by Malays in their own villages.

As will be seen from the table, of the twelve fowls employed for this experiment all remained healthy with the exception of No. 12 which on the 42nd day of the experiment developed polyneuritis. The attack ran the usual course and on emulsion of rice polishings and padi the fowl recovered completely in 6 weeks.

In the second experiment in this series (Experiment No. 32) which was one of a group of experiments to which particular attention will be directed later Malay rice was prepared from a partially husked padi imported from Indo-China.

The method of preparation of this rice was similar to that employed in Experiment No. 31.

As the supply of this product was limited only eight fowls were employed for this experiment and it lasted only five weeks. All the fowls remained normal throughout this period.

The occurrence of a case of polyneuritis in a fowl fed on "Malay" rice calls for some comment. It is the single instance throughout these experiments in which hundreds of fowls have been employed, in which polyneuritis developed in a fowl on a diet other than white polished rice. The clinical appearances were in all respects identical with those of other cases of polyneuritis and we do not assign to the case an origin other than dietary. It appears that even the limited amount of polishing to which Malay rice is subjected in its preparation may on occasion be harmful.

Experiment No. 31.—Malay Rice prepared from Malay padi.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	——
1	Red cock	1542	1355	1465	1410	1332	1440	1315	
2	Brown hen	1282	1487	1425	1450	1443	1430	1440	
3	Red cock	1348	1295	1250	1272	1295	1300	1264	
4	Black and white hen ..	1145	1270	1210	1282	1250	1215	1160	
5	Brown hen	1182	1185	1185	1162	1197	1240	1260	
6	Yellow and black hen ..	902	933	948	915	907	828	837	
7	Black hen	1225	1262	1295	1333	1317	1335	1352	
8	Black hen	1130	1125	1180	1185	1185	1140	1170	
9	Black hen	1145	1295	1148	1180	1230	1235	1262	
10	Black hen	1302	1235	1275	1270	1265	1245	1260	
11	Red cock	1180	1170	1172	1180	1190	1142	1202	
12	Brown hen	1140	1093	1085	1030	885	780	678	Developed polyneuritis 7th week.

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
7.2	0.63	77.29	14.	0 88

$P_2 O_5$

0.37

Experiment No. 32.—Malay Rice prepared from "Cargo Rice."

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week	——
1	Yellow hen	1045	1062	1092	1070	1090	1095	Healthy throughout.
2	Yellow cock	1685	1685	1705	1680	1695	1700	,, ,,
3	Brown hen	1425	1409	1383	1357	1367	1385	,, ,,
4	Brown hen	1525	1676	1675	1525	1475	1505	,, ,,
5	Brown cock	1660	1730	1740	1710	1690	1720	,, ,,
6	White hen	775	769	730	735	748	755	,, ,,
7	Brown hen	940	980	965	917	942	990	,, ,,
8	Brown hen	1210	1225	1300	1327	1330	1405	,, ,,

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
7·7	0.23	75·43	15·5	1.14

$P_2 O_5$

0.52

Experiments with Polishings.

As it now appeared that the harmfulness of a rice diet varied directly as the extent to which the rice had been milled, it was determined to test this hypothesis further by adding to a white polished rice, which when given alone had been shown to be harmful, a quantity of polishings equal in amount to that which had been milled away. The miller estimates that from 40 parts of padi there are produced 25 parts of rice and 5 parts of polishings; 16% of the husked grain is thus lost in polishing. An actual weighing of some thousands of grains of unpolished and polished rice from the same lot of padi showed that the loss was about 10%.

In the first experiment of this series, Experiment No. 33, the white polished rice selected was the same sample as that employed in Experiment No. 15, and which when fed alone had resulted in six cases of polyneuritis among twelve fowls in five weeks.

The rice polishings were from the same sample. They were sifted in order to remove husks and broken rice and given in the form of an emulsion with distilled water.

During the first and second weeks of the experiment an amount of emulsion equal to 10 grammes of polishings was given daily for a fowl eating 60 grammes of rice.

During the 3rd week 8 grammes of polishings were given daily, during the 4th and 5th weeks 6 grammes, during the 6th week 4 grammes and during the 7th week 3 grammes were given.

Up to the 7th week all the fowls gained slightly in weight. In the 7th week there was a moderate loss amounting to 2.6 grammes per kilogramme of body weight.

In the 7th week there was a moderate loss 2.6 grammes per kilo. It was concluded therefore that the amount of polishings necessary to add to 60 grammes of this sample of white polished rice to maintain the normal nutritive equilibrium was between 3 and 4 grammes, say 3.5 grammes. This amount being added there was in the eighth week a slight gain in weight in the whole group.

It was considered desirable to repeat this result with a rice from a beri-beri outbreak. An experiment was therefore planned in which eight fowls were fed on the Durian Tipus rice, each alternate fowl receiving in addition an amount of polishings equal to 3.5 grammes per kilo of body weight.

The result is as shown by the Experiment No. 34. In four weeks two of the fowls fed on rice alone had developed polyneuritis and all four had lost weight while the four fowls which were given polishings in addition remained healthy and at the conclusion of the experiment all had gained in weight.

The conclusion was now arrived at that certain essential substances are lacking in the case of white polished rice and that the addition of rice polishings to a diet of white polished rice prevents the occurrence of polyneuritis in fowls.

Experiment No. 33.—White Polished Rice + Polishings.

No.	Description.	Original wt	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.
1	Yellow cock	1402	1380	1375	1362	1385	1365	1320	1235	1240
2	Black hen	655	670	655	648	652	635	635	620	580
3	Red hen	870	882	890	902	925	980	1005	1010	1030
4	Brown hen	672	745	740	745	745	737	735	700	692
5	White hen	693	730	770	780	792	770	782	735	717
6	Yellow hen	1262	1340	1345	1390	1400	1420	1420	1352	1372
7	Grey hen	752	772	805	818	772	815	818	795	770
8	Brown hen	1648	1655	1620	1612	1615	1590	1605	1525	1585
9	Yellow cock	2015	2101	2154	2170	2193	2137	2104	2095	2044
10	Red cock	1437	1500	1510	1518	1525	1540	1548	1550	1580
11	White cock	1370	1372	1372	1373	1387	1378	1398	1385	1440
12	Red cock	1355	1410	1440	1350	1462	1505	1525	1540	1565

	Protein.	Fat.	Carbohydrate.	Moisture.	Ash.	$P_2 O_5$
White polished rice	7.3	0.40	77.43	14.3	0.57	0.28
Polishings	11.7	10.0	59.05	11.95	7.3	4.6

EXPERIMENT No. 34.—Nos. 1, 3, 5, 7 White Polished Rice + Polishings Nos. 2, 4, 6, 8, White Rice only.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week
1	Black hen	1237	1309	1380	1398	1383
2	Red cock	1254	1200	1130	1047	Polyneuritis
3	White cock	1625	1615	1625	1635	1630
4	Brown hen	890	875	820	827	778
5	White hen	875	865	900	900	900
6	White cock	1810	1648	1460	1162	Polyneuritis
7	Yellow hen	918	970	991	1030	1040
8	Brown hen	1005	965	895	817	820

	Protein.	Fat.	Carbohydrate.	Moisture.	Ash.	$P_2 O_5$
White Rice (D.T.)	7.45	0.17	78.02	13.85	0.51	0.28
Polishings	11.7	10.0	59.05	11.95	7.3	4.6

The Phosphorous Content of Rice as an indicator of the extent to which rice has been polished.

Having proved that the occurence of polyneuritis in fowls and of beri-beri in man is due to the consumption of rice from which the subpericarpal layers have been removed by the process of polishing, that such rice is equally harmful whether freshly prepared or stored for months, the development in it of a poison is excluded and it is necessary to revert to the consideration of the results obtained from analyses of rices.

In the tables the results of these analyses are given both for the undried and dried materials. The table showing the results calculated on the dried materials facilitates comparison.

Comparing the diet unpolished rice with that of polished rice it will be seen that there are considerable differences. A diet of polished rice made up with polishings to the necessary amount for the maintenance of weight and health approximates in composition to one of unpolished rice, and it might therefore be inferred that the deficiency of white rice in respect of proteins, fats, and salts was accountable for its harmful results. The protein content of polished rice differs only by a slight amount from that of two parboiled rices which were proved harmless and allowing for experimental errors the differences are apparently insufficient to account for the results. In respect of fat the polished rice is poorer but the difference is a small one and, as has been previously stated, can hardly account for the difference in the results. The ash gives a very imperfect representation of the salts present but there is a considerable difference in the amount of ash present in the unpolished and polished rices yet even the amount present in the latter rice exceeds that obtained from one of the parboiled rices.

Now in the case of parboiled rice extracted with hot alcohol (94%) the amount of fat removed is relatively great but of proteins and ash relatively small and in the case of parboiled rice extracted with proof spirit similar results are observed.

The proteins were invariably estimated by the Kjeldahl process and allowing for the possibilities of experimental error it must be admitted that the differences in respect of proteins as determined by this method will not explain the results.

The question of the carbohydrates cannot seriously be considered as these were calculated by difference.

These methods of analysis failed in every way to explain the results and it was necessary to employ other methods. It was suggested to one of us by Dr. Mott that the deficiency of white rice in lipoids might be of importance and various attempts were made to determine the amount of these present in rices but the process was a tedious one and the results not satisfactory. It was decided therefore to estimate the Phosphorus Pentoxide content of the various rices.

Estimation of Phosphorus Pentoxide (P_2O_5)

Two methods were employed for the conversion of the phosphorus to inorganic combinations.

By the first process—

1. A weighed quantity of the material was burned in a platinum basin and the complete combustion of the organic matter was facilitated by sprinkling pure Potassium Nitrate over the ash.

2. The residue was treated with 10 c.c. of strong Hydrochloric Acid and evaporated to dryness over a water bath.

3. The residue was heated with 5 c.c. strong Hydrochloric Acid and 50 c.c. of distilled water for five minutes.

4. Filtered and the filter-paper washed with hot water till the washings ceased to give a precipitate with Silver Nitrate and Nitric Acid.

5. The combined filtrate and washings were evaporated to about 50 c.cm.

By the second process—

1. A weighed quantity of the material was placed in a Kjeldahl flask and heated with concentrated Sulphuric Acid and Potassium Hydrogen Sulphate until the fluid became colourless.

2. The fluid was diluted with water and filtered. The filter-paper was washed with water until the washings ceased to give a precipitate with Barium Chloride.

3. The combined filtrate and washings were evaporated to about 50 c.cm.

4. The concentrated filtrate and washings obtained either by the first process or the second process were neutralized with Ammonium Hydrate and a drop or two of Nitric Acid added to clear.

5. To the solution 10 grammes of Ammonium Nitrate were added, the solution warmed to about 40°C and excess of Molybdic solution added.

6. The mixture was kept in a warm place overnight and in the morning filtered. The precipitate was washed with a cold solution of Ammonium Nitrate (5%).

7. The Phospho-molybdate precipitate was dissolved in hot water and Ammonium Hydrate, it was so arranged

that the filtrate and washings measured 100 c.c. or thereabouts.

8. The solution of Phospho-molybdate was neutralized with Hydrochloric Acid and excess of Magnesia Mixture added. After half an hour 30 c.c. of Ammonium Hydrate (10%) were added.

9. The mixture was allowed to stand overnight. In the morning it was filtered and the precipitate washed with Ammonium Hydrate (2.5%) till free from chloride.

10. The precipitate was dried, ignited and the residue of Magnesium Pyrophosphate ($Mg_2\ P_2O_7$) weighed. From this weight the amount of Phosphorus Pentoxide was calculated by the use of the factor 0.6394.

By whichever method the conversion of the phosphorous into inorganic combination is effected the results obtained are the same and it is entirely a matter of individual choice which shall be selected.

The gravimetric process was employed in preference to the volumetric because of its greater accuracy and now that the facts have been determined there is no reason why the volumetric process should not be employed.

Nearly two hundred estimations have been made and the results were invariably recorded on the undried material. The percentage of moisture in various kinds of rice does not vary greatly, being usually from 13—14% or thereabouts, and it was found that the percentage calculated on the dried material did not yield any information other than that obtained from that calculated on the undried material.

Polished white rice of the kind commercially known as Siam rice yields on an average 0.26% of Phosphorus Pentoxide and

is the variety usually associated with severe outbreaks of beri-beri. Schaumann gives an analysis of a rice yielding only 0.1% Phosphorous Pentoxide. This is lower than any result obtained here.

Polished white rice of the kind commercially known as Rangoon rice yields on an average 0.328% of Phosphorus Pentoxide and the incidence of beri-beri is less on this kind of rice than the former.

Malay rice yields on an average 0.38% of Phosphorus Pentoxide and the incidence of beri-beri is still less on this kind of rice.

Parboiled rice yields on an average 0.415% or over of Phosphorus Pentoxide and beri-beri does not occur when this kind of rice is eaten.

Unpolished rice yields on an average 0.54% of Phosphorus Pentoxide and can never produce beri-beri.

The great increase in the consumption of parboiled rice has induced the local millers to improve the appearance of their product and this result some of them have sought to achieve by polishing or as it is technically called "pearling." Attention has previously been directed to the difficulty of accomplishing this on account of the toughening of the grain by soaking and steaming but by the use of stone polishers it is possible to remove a considerable amount of the subpericarpal layers with consequent diminution of the Phosphorus Pentoxide content. We recently examined such a rice which only yielded 0.34% of Phosphorus Pentoxide (see Experiment 30).

Another sample of parboiled rice after hulling was examined and yielded 0.6% of Phosphorus Pentoxide. After pearling once it yielded 0.5% and after pearling twice it yielded 0.4%.

An unpolished rice yielding 0.56% of Phosphorus Pentoxide was polished in the ordinary way and the polished or white rice yielded only 0.26%.

AVERAGE RESULTS OF ALL ESTIMATIONS.

	Percentage of P_2O_5
Polished rice (Siam)	0.26
Polished rice (Rangoon)	0.328
Malay rice	0.38
Parboiled rice	0.415
Unpolished rice	0.54

Fowls fed on polished rice and receiving polishings in sufficient amount are receiving a dietary which approximates in its content of Phosphorus Pentoxide to a diet of unpolished rice. The harmfulness of rice is therefore in inverse proportion to its Phosphorus Pentoxide content and in direct proportion to the extent to which it has been polished.

None of the rices connected with outbreaks of beri-beri yielded more than 0.26% of Phosphorous Pentoxide. The rices substituted for these and which were effective in preventing the continuance of the outbreaks yielded not less than 0.4% of that substance.

Attention should be directed to the percentage of the Phosphorus Pentoxide in parboiled rice after extraction with 94% alcohol, the diminution in amount is extremely small and apparently negatives the vital importance of this substance. In practice extracted rice would never be under consideration and the value of this estimation as an indicator of the extent to which rice has been polished has stood the test of numerous experiments.

It will be contended that the estimation of any of the other constituents of rice would serve equally well for this purpose. It has already been shown that the estimation of

proteins is not satisfactory. The removal of the total fats from a rice is an extremely tedious process and the determination of total ash is complicated with errors of volatilization and incomplete ashing.

Admitting the .value of those other estimations it must still be conceded that the estimation of Phosphorus Pentoxide permits of a reasonable margin of error and furnishes differences which can be accurately determined and are more striking than would be furnished by any of the other constituents.

ANALYSIS OF RICES.

—	——	Protein.	Fat.	Carbo-hydrates.	Ash.	Moisture.	Percentage of Phosphorus Pentoxide.	Effect on fowls: estimated by the occurrence of polyneuritis.
1	Polishings	13.7	14.16	52.77	7.54	11.83	4.1	
2	Unpolished rice	9.0	1.65	75.52	1.08	12.75	0.56	—
3	Polished rice	8.6	0.22	76.23	0.6	14.35	0.26	+
4	Polished rice (washed)	8.2	0 22	75.04	0.34	16.2	0.21	+
5	Polished rice (washed) plus polishings ..	8.61	1.29	73.35	0.89	15.86	0.463	—
6	Parboiled rice	7.55	0.45	77.76	0.94	13.3	0.427	—
7	The same rice after extraction with 94% alcohol	7.6	0.06	80.66	0.88	10.8	0.425	+
8	Parboiled rice	7.8	0.5	76.62	0.78	14.3	0.41	—
9	The same rice after extraction with proof spirit	7.65	0.10	80.01	0.44	11.8	0.29	+
10	Siam rice (Depot)	7.8	0.15	77.49	0.56	14	0.28	+
1	Siam rice (D. T.)	7.45	0 17	78.02	0 51	13.85	0.28	+
2	Rangoon rice	7.3	0.63	77.15	0.74	14 18	0.33	+
3	Indian rice	6.3	0.2	78.31	0.84	14.35	0.345	—
4	Malay rice from cargo rice	7.7	0.23	75.43	1.14	15.5	0.52	—
5	Malay rice from Malay padi	7.2	0.63	77.29	0.88	14.0	0.37	+
6	Siam rice No. 2 quality stale	7.3	0.40	77.43	0.57	14.3	0.28	+
7	Siam rice No. 2 quality freshly milled ..	8.1	0.41	76.76	0 47	14.26	0.27	+
8	Siam rice No. 1 quality	6.9	0.20	77.26	0.40	15.24	0.22	+

Analysis of Rices (Calculations Based on Dried Materials).

—	—	Protein.	Fat.	Carbo-hydrates.	Ash.	Percentage of Phosphorus Pentoxide.	Effect on fowls: estimated by the occurrence of polyneuritis.
1a.	Polishings	15.5	16.0	60.0	8.5	4.6	
2a.	Unpolished rice	10.3	1.89	86.58	1.23	0.64	—
3a.	Polished rice	10.0	0.25	89.05	0.7	0.3	+
4a.	Polished rice (washed)	9.7	0.26	89.64	0.4	0.25	+
5a.	Polished rice (washed) plus polishings	10.2	1.53	87.22	1.05	0.55	—
6a.	Parboiled rice	8.7	0.51	89.79	1.0	0.492	—
7a.	The same rice after extraction with 94% alcohol	8.5	0.06	90.46	0.98	0.476	+
8a.	Parboiled rice	9.1	0.58	89.76	0.56	0.47	—
9a.	The same rice after extraction with proof spirit	8.6	0.11	90.79	0.5	0.32	+

The results of numerous experiments had now made it clear that the addition of rice polishings to a diet of white polished rice prevented the harmful effects of such a diet. The further researches consisted in an endeavour to determine the nature of the substances in rice polishings which were responsible for this result.

In order that the results in the various experiments of the series should be comparable it was considered desirable that the various rices and rice products employed should be derived from the same lot of pádi. Accordingly we obtained from a rice mill in Singapore the materials desired:—

(1) A quantity of the rice as it passed from the huller to the polishing machines. At this stage the grain is deprived of the husk only (unpolished rice).

(2) A quantity of the rice after having passed through the polishing machines (white polished rice).

(3) A quantity of the rice polishings or rice meal collected from the polishing machines.

A preliminary series of experiments was carried out to test the value of these various foodstuffs when fed to fowls.

In Experiment No. 35 the unpolished rice was employed. It was shown that this foodstuff in addition to which only water was supplied sufficed to maintain fowls in good health for many weeks.

In Experiment No. 36 the white polished rice was employed. Within three weeks six fowls out of a total of twelve fowls included in the experiment suffered from polyneuritis.

In Experiment No. 37 the white polished rice diet identical with that in Experiment No. 36 was employed. In addition each fowl received daily 5 grammes of sifted rice polishings emulsified in water. All the fowls remained healthy.

This series of experiments confirmed results previously obtained and showed that these materials were suitable for employment in the further researches which it was proposed to undertake.

As an instance of the practical application of these principles, Dr. J. M. Atkinson, Principal Civil Medical Officer, Hongkong permits us to refer to some notes he has made in regard to the medical history of the Victoria Gaol, Hongkong. At the meeting of the Far Eastern Association of Tropical Medicine at Manila in March 1909 we communicated the results of our observations in regard to the causation of beri-beri and advanced the view that the use of unpolished rice would be found an effective preventive measure. Dr. Atkinson who was the official representative of Hongkong at the meeting, on his return to the Colony made inquiries as to the diets in the various public institutions. He found that in the Victoria Gaol which had been singularly free from beri-beri, unpolished rice had been in use for many years. The average daily number of prisoners in this Gaol is over 500, many of them being long sentence prisoners.

The Chief Superintendent of Police reported the facts as follows.

"No case of beri-beri has originated in the jail in the last fifteen years. Polished rice is not supplied to the prisoners and never has been. They have always been given unpolished rice as it is cheaper than polished rice."

As beri-beri is one of the principal diseases afflicting the native population of Hongkong this observation is of great

importance as showing the value of an unpolished rice diet in preventing beri-beri.

M. Breaudat and Dr. Denier of the Pasteur Institute Saigon have recently published the report of an experiment, the results of which showed the value of rice polishings (*son*) as a preventive of human beri-beri.

Experiment No. 35.—Unpolished Rice.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week	6th week.	7th week
1	Red cock	1380	1380	1380	1370	1380	1370	1395	1420
2	Red cock	1145	1195	1205	1205	1220	1255	1280	1310
3	Black cock	1080	1115	1170	1180	1180	1125	1160	1180
4	Yellow hen	1035	1090	1100	1072	1070	1040	1020	1040
5	Black cock	1145	1200	1280	1305	1370	1345	1320	1340
6	Yellow hen	1450	1460	1330	1305	1345	1270	1275	1240
7	Black hen	925	920	930	935	925	940	960	940
8	Yellow cock	1185	1105	1175	1265	1330	1300	1325	1285
9	Brown hen	1640	1660	1430	1350	1360	1415	1390	1380
10	Red cock	1405	1450	1450	1475	1475	1340	1390	1430
11	Yellow hen	1030	980	990	940	995	980	980	995
12	Brown cock	1020	1060	1060	1035	1030	1035	950	1050

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
9.0	1.65	75.52	12.75	1.08

$P_2 O_5$
0.56

Experiment No. 36.—White polished Rice (washed).

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.
1	Black cock	1175	1045	942	810
2	Black hen	1205	1115	1082	Polyneuritis
3	Red cock	1300	1250	1165	Polyneuritis
4	Brown hen	1595	1470	1370	1255
5	Yellow hen	1390	1210	1098	985
6	Black hen	1025	990	935	795
7	White hen	1180	1175	1060	940
8	Yellow hen	1470	1395	1275	1120
9	Black hen	1210	1150	1115	Polyneuritis
10	Brown hen	1370	1190	1025	Polyneuritis
11	Red cock	1950	1810	1635	Polyneuritis
12	White cock	1790	1720	1670	Polyneuritis

Protein.	Fat.	Carbohydrate.	Moisture.	Ash.
8.2	0.22	75.04	16.2	0.34

$P_2 O_5$

0 21

Experiment No. 37.

White Polished Rice (washed) + Rice Polishings.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Brown cock	1390	1470	1490	1480	1560	1575
2	White hen	1470	1560	1470	1320	1400	1430
3	Black hen	1340	1410	1420	1380	1400	1385
4	Red cock	1505	1505	1510	1490	1525	1505
5	White hen	1720	1730	1630	1515	1605	1575
6	Brown hen	1215	1275	1300	1420	1445	1325
7	Black hen	1275	1350	1380	1240	1340	1390
8	Red cock	1925	1990	1955	1910	1945	1930
9	Black hen	1250	1230	1200	1120	1150	1100
10	White hen	1580	1530	1470	1485	1535	1530

	Protein.	Fat.	Carbohydrate.	Ash.	Moisture.	$P_2 O_5$
White polished Rice washed	8.2	.22	75.04	0.34	16.2	.21
Polishings	13.7	14.16	52.77	7.54	11.83	3.55

The Effect of Exposure to High Temperatures.

At this point the various methods of analysis suitable for the recognition of the physiologically active substances were reviewed. The methods hitherto employed for the recognition of specific differences among the various rices and rice products experimented with had always involved the exposure of these substances to high temperatures.

Bearing in mind the results obtained by Grijns in his experiments with kachang idju (Geneeskundig Tijdschrift voor Nederlandsch Indie 1901) and by Holst and Fröhlich (18) with various meats and vegetables, it was decided to test the effect of exposure to high temperatures on the materials with which we were working.

The first substance experimented with was the rice polishings. Quantities of polishings sufficient for six fowls for one day were mixed with water in flasks and heated in the autoclave for one hour at 120°C. In Experiment No. 38 a group of fowls on a white polished rice diet were each given daily a portion of the emulsion so prepared equivalent to five grammes of polishings. No cases of polyneuritis appeared among them in the five weeks during which the experiment was in progress.

In the next experiment, No. 39, polishings heated for two hours at 120°C were employed. One fowl died in the third week of the experiment without showing the clinical signs of polyneuritis or the characteristic nerve changes on post-mortem examination.

This unexpected result caused us to initiate further experiments to determine whether physical conditions apart from the temperature were responsible for this result.

In Experiment No. 40, padi which had been sterilized in a small bag suspended in the wire basket of the autoclave for one hour at 120°C was employed. One group of fowls Nos. 1 to 6 were fed on this and another group Nos. 7 to 12 were fed on the untreated padi. This experiment gave a result identical with that of other observers.

In Experiment No. 41, unpolished rice was employed. Nos. 1 to 4 were fed on unpolished rice untreated. Nos. 5 to 8 were fed on unpolished rice sterilised in a porcelain basin in the autoclave for one hour at 120°C. Nos. 9 to 12 were fed on unpolished rice sterilised for two hours in the same way. The results of these experiments showed that when sterilised in an atmosphere of steam for one or two hours at 120°C the physiological activity of the protective substances was destroyed.

In Experiment No. 42, padi immersed in water in a porcelain basin and sterilised in a autoclave for one hour at 120°C was employed. The fowls remained healthy.

In Experiment No. 43 padi sterilised for one hour at 120°C in a hot-air steriliser was employed. The fowls remained healthy.

In certain large institutions rice is cooked by steam under pressure. By this method larger quantities are dealt with than by cooking in open vessels and the cooking is presumably more quickly carried out. An opportunity of making a practical test of the relative merits of these two methods of cooking we owe to the courtesy of Dr. Gray and Dr. Freer.

In Experiment No. 44 a group of fowls was fed on parboiled rice cooked in the usual way in open vessels. All the fowls remained healthy.

In Experiment No. 45 the same rice cooked by steam under pressure as issued to the inmates of a large institution

EXPERIMENT No. 38.—White Polished Rice + Polishings in emulsion heated at 120°C for one hour.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	White cock	1330	1400	1445	1463	1455	1485
2	Brown cock	1200	1245	1260	1220	1220	1195
3	Black cock	1345	1380	1375	1405	1415	1400
4	Yellow cock	1265	1170	1120	1180	1160	1065
5	Black cock	1420	1400	1515	1495	1515	1510
6	Black cock	1535	1515	1535	1510	1465	1475

Experiment No. 39.

White Polished Rice + Polishings in emulsion heated at 120°C for two hours.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	——
1	White cock	1485	1430	1340	1370	1380	1340	
2	Brown cock	1195	1175	1090	1060	1050	1035	
3	Black cock	1400	1420	1430	1440	1460	1405	
4	Yellow cock	1065	1015	870	Died			No degeneration of nerves.
5	Black cock	1510	1465	1400	1390	1360	1340	
6	Black cock	1475	1460	1435	1410	1410	1415	

EXPERIMENT No. 40.—Nos. 1—6 Untreated Padi. Nos. 7—12 Padi heated at 120°C for two hours.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Yellow cock	1140	1210	1175	1140	1135	
2	Speckled cock ..	1500	1500	1545	1400	1420	
3	Red cock	1600	1640	1660	1600	1625	
4	Yellow cock	1340	1340	1310	1310	1370	
5	Red cock	1505	1550	1575	1555	1550	
6	Yellow cock	1295	1340	1375	1340	1325	
7	Black cock	1540	1555	1535	1495	1375	
8	White cock	1715	1680	1630	Polyneuritis		
9	Speckled cock ..	1540	1520	1490	1420	Polyneuritis	
10	Yellow cock	1710	1680	1715	1485	Polyneuritis	
11	White cock	1240	1200	1110	Polyneuritis		
12	Red cock	1400	1360	1340	1240	Polyneuritis	

EXPERIMENT No. 41.—Nos. 1—4 Unpolished Rice.

Nos. 5—8 Unpolished Rice sterilised one hour.

Nos. 9—12 Unpolished Rice sterilised two hours.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	Yellow cock	1230	1100	1255	1210	1240
2	Brown cock	1075	1075	1140	1165	1155
3	White cock	1460	1490	1325	1485	1530
4	Brown cock	1355	1350	1205	1280	1305
5	White cock	1410	1305	1150	1005	
6	Brown cock	1250	1060	890	830	
7	White cock	1290	1300	1020	Polyneuritis	
8	Brown cock	1225	1125	950	855	Polyneuritis
9	White cock	1125	1065	945	Polyneuritis	
10	Brown cock	1170	1080	1000	Polyneuritis	
11	Black cock	1365	1365	1160	1045	
12	Brown cock	1045	975	870	840	Polyneuritis

Experiment No. 42.

Padi submerged in water and sterilised at 120°C.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Yellow cock	1240	1200	1220	1205	1275	1305
2	Brown cock	1155	1165	1170	1180	1170	1180
3	Black cock	1530	1520	1535	1420	1505	1510
4	Brown cock	1305	1280	1270	1295	1285	1325
5	White cock	1425	1395	1410	1450	1455	1425
6	Black cock	1340	1250	1200	1335	1320	1295

EXPERIMENT No. 43.—Padi sterilised 120°C Dry heat.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Red cock	1250	1230	1235	1250	1235	1220
2	Speckled cock ..	1560	1590	1560	1560	1530	1515
3	Red cock	1250	1210	1225	1235	1220	1220
4	Grey cock	1435	1370	1372	1375	1365	1355
5	Black cock	1775	1775	1880	1950	1975	1965
6	Black cock	1535	1455	1460	1500	1535	1555

EXPERIMENT No. 44.—Parboiled Rice cooked in open vessel.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	White cock	1295	1200	1145	1180	1200	1235
2	White cock	1110	1035	1070	1080	1120	1140
3	Grey cock	1235	1190	1155	1140	1220	1170
4	Black cock	1075	1030	980	940	1055	980
5	White cock	1335	1510	1515	1510	1560	1695
6	Black cock	1135	1115	1015	1110	1110	1085

EXPERIMENT No. 45.—Parboiled Rice cooked by steam under pressure.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	White cock	1300	1185	1055	940	1000	855
2	Black cock	1030	930	845	760	Polyneuritis	
3	Red cock	1070	1105	915	810	810	670
4	Brown cock	1220	1200	1165	1055	960	Polyneuritis
5	Yellow cock	1230	1140	1110	970	950	890
6	White cock	1120	975	825	670	Illness. Not Polyneuritis.	

was fed to a group of fowls. The cooking process involved exposure to steam under a pressure of two atmospheres at 120°C or thereabouts for two and a half hours. The fowls in this group rapidly lost weight and cases of polyneuritis developed among them.

These experiments showed that physical conditions other than temperature influenced the result, immersion in water for example in the case of padi seemed to negative entirely the destructive effect of an atmosphere of steam under the conditions which obtain in an autoclave. This series of experiments, has not been carried to a conclusion, but there is evidence that the destructive effect of high temperatures is complicated by the consideration of other physical conditions.

It was evident however that methods of analysis involving exposure of the materials to high temperatures would not enable us to determine the nature of the substances for which we sought and recourse was had to other methods.

Experiments to Isolate the Protective Substances contained in Rice Polishings.

From this point researches were carried out for the isolation of the substance or combination of substances in polishings which were responsible for this result.

For the purpose of testing the value of the various materials fowls weighing 1200 grms. or thereabouts were employed. Each, as in previous experiments, was confined in a separate cage. The fowls received rice twice daily at 10 a.m. and 3 p.m. and when receiving polishings or materials prepared from polishings the substance in question was given as an emulsion by means of a stomach-tube half an hour after the rice had been given. Every fowl was weighed once a week at 12 noon.

As the result of a series of observations it had been determined that fowls weighing from 1200 to 1400 grms. required about 60 grms. of unpolished rice daily and, if fed on 60 grms. of the polished rice used in these experiments they required in addition 5 grms. of sifted polishings for the maintenance of weight and health.

In a previous experiment where products derived from different lots of padi were employed, 3.5 grms. of the polishings were shown to be sufficient with the white-rice then in use. In the present experiment all the products employed unpolished rice, polished rice, polishings, etc., were derived from the same lot of padi.

For purposes of comparison the following results of analyses are given :—

	Protein.	Fats.	Carbo-hydrates.	Ash.	Moisture.	Percentage of P_2O_5.
Polishings (sifted)	13.7	14.16	52.77	7.54	11.83	4.1
Unpolished rice	9.0	1.65	75.52	1.08	12.75	0.56
Polished rice	8.6	0.22	76.23	0.6	14.35	0.26

When the composition of these articles is calculated on dried materials the differences are rendered more striking and accurate, and when in a similar manner the composition of a diet made up of 60 grms. of polished rice and 5 grms. of polishings is calculated it will be seen how closely such a diet approximates to one of unpolished rice.

CALCULATED ON DRIED MATERIALS.

	Protein.	Fats.	Carbo-hydrates.	Ash.	Percentage of P_2O_5
Polishings (sifted)	15.5	16.0	60 0	8.5	4.65
Unpolished rice	10.3	1.89	86.58	1.23	0.64
Polished rice	10.0	0.25	89.05	0.7	0.3
Ration 60 grams. polished rice plus 5 grams. polishings contains per cent	10.4	1.5	86.8	1.3	0.64

Sifted polishings were invariably employed because polishings as received from the millers contain a considerable admixture of husk and broken rice.

Polishings when fresh are neutral in reaction but on keeping they become acid. This change does not impair their efficiency however and polishings which have been stored with ordinary care for months are quite as valuable as the fresh materials. The ordinary process of cooking does not impair the value of polishings. For these reasons it is considered that the essential substance or substances are not unstable.

Experiments to determine the value of Fat in Polishings.

Fat in the rice-grain is mostly confined to the subpericarpal layers. Unpolished rice is therefore richer in fat than polished rice and polishings are very rich in fat.

To determine the value of this fat a quantity of sifted polishings was packed in a percolator and percolated repeatedly with Petroleum Ether. In this way the amount of fat in the polishings was reduced from 14.16% to 0.6%. The fat-free polishings were dried by exposure to the sun until free from Petroleum Ether.

Twelve fowls were fed on polished rice and received in addition daily 4.5 grms. of fat-free polishings, being the approximate equivalent of 5 grms. of sifted polishings (Experiment No. 46). The fowls remained healthy and maintained their weight just as had been the case when fowls received polished rice and sifted polishings. The non-importance of fat was therefore decided and its exclusion from the number of possibilities was of the utmost value since the fat had hitherto complicated our experiments.

Experiments with the Substances Soluble in 0·3% Hydrochloric Acid.

Estimations of the percentage of Phosphorus Pentoxide in rices had consistently shown their value as indicators of the liability or otherwise of a given rice to produce polyneuritis. Thus the higher the percentage of Phosphorus Pentoxide contained in a rice the less liable was that rice to produce polyneuritis when fed to fowls.

The unpolished rice employed contained 0.56% Phosphorus Pentoxide and did not cause polyneuritis. The polished rice contained 0.26% Phosphorus Pentoxide and invariably caused polyneuritis, while washed rice containing 0.22% Phosphorus Pentoxide was more harmful than the unwashed polished rice. This suggested the probability that the essential substance was one containing Phosphorus.

Now it was known that a large percentage of the Phosphorus compounds present in rice polishings were soluble in 0.3% Hydrochloric Acid.

An experiment was therefore carried out to determine if when polishings were treated with 0.3% HCl, the physiologically active substances were removed.

Polishings in quantities of 180 grms. being the amount required by twelve fowls in three days, were mixed with 1000 cc. 0.3% Hydrochloric Acid, stirred during the day and

Experiment No. 46.

White Polished Rice washed + Polishings fat free.

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Black hen	1510	1525	1340	1360	1395	1360
2	White cock	1225	1230	1220	1215	1235	1255
3	Red cock	1700	1840	1900	1855	1895	1860
4	Brown hen	1215	1220	1305	1310	1340	1295
5	Red cock	1550	1515	1460	1430	1460	1450
6	Black cock	1465	1520	1500	1485	1520	1530
7	Brown hen	1445	1420	1290	1335	1330	1305
8	White hen	1685	1650	1750	1755	1745	1665
9	Brown hen	1225	1195	1195	1085	1070	1035
10	Red cock	1300	1350	1390	1380	1370	1380
11	Black hen	1265	1235	1220	1220	1230	1235
12	White cock	1410	1410	1390	1390	1380	1340

	Protein.	Fat.	Carbohydrate.	Ash.	Moisture.	$P_2 O_5$
White Polished Rice washed	8 2	.22	75.04	0.34	16.2	.21
Polishings Fat free		.67				

the following morning filtered through a Buchner's filter. 100 cc. of 0.3% Hydrochloric Acid were used to wash out the vessels. When fluid could no longer be extracted from the mass it was mixed with 600 cc. of 0.3% Hydrochloric Acid stirred during two hours and thereafter filtered as before.

The extracted polishings were mixed with distilled water, nearly neutralized with Sodium Carbonate, and the volume adjusted to 1080 cc. 30 cc. of this emulsion contained 5 grms. of polishings less the materials dissolved out by the acidulated water.

The combined filtrates obtained from 180 grms. of polishings were nearly neutralized with Sodium Carbonate and concentrated at a low temperature to a volume of 1080 cc. 30 cc. of this suspension contained the substances solved out by acidulated water from 5 grms. of polishings.

Twelve fowls were obtained and fed on washed polished rice, each receiving daily 30 cc. of the emulsion of extracted polishings (Experiment No. 47). Cases of polyneuritis occurred. This experiment was repeated (Experiment 48) with similar results.

Twelve fowls were fed on washed polished rice each receiving in addition 30 cc. of the suspension of dissolved substances (Experiment No. 49). Cases of polyneuritis did not occur.

When 100 grammes of polishings are extracted in the manner described 26 grammes of solids pass into solution. In this 26 per cent. therefore of the original polishings are contained the physiologically active substances.

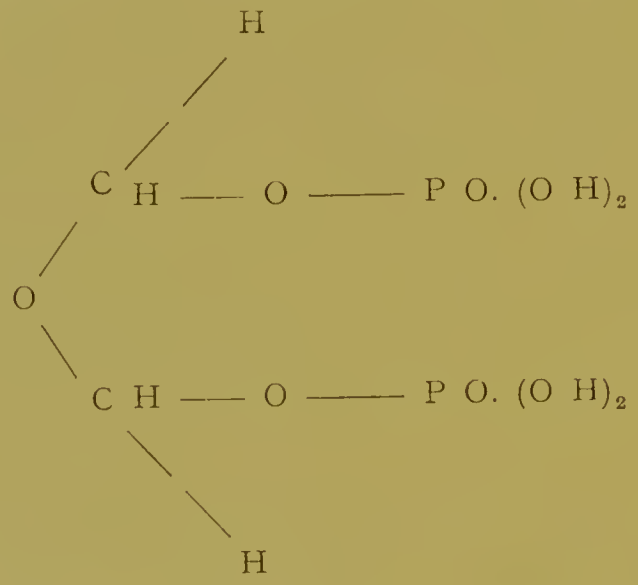
Experiments with Phytin.

It having thus been made clear that the substances of physiological importance in polishings were removed unchanged

by extracting with 0.3% HCl, the further research consisted in division of this fraction by various methods.

Of the substances contained in rice polishings which are soluble in 0.3% Hydrochloric Acid an important constituent is the phosphorus compound Phytin. Dr. Hans Aron has claimed that Phytin is of value in preventing the onset of polyneuritis in fowls fed on a white rice diet. The results of Dr. Arons experiments with this compound do not appear however to justify the claims that he has made for it.

Phytin was first isolated by Posternak from plant-seeds and the structural formula stated by him to represent phytic acid is



Phytin is stated to be the Calcium Magnesium salt of this acid and assuming the four hydroxyl groups to be replaced by one atom each of Calcium Magnesium, the salt would contain 8 05% of Magnesium and 13.4% of Calcium. These are lesser amounts than those recorded by Contardi who by actual analysis found 8.97% of Magnesium and 13.8% of Calcium.

The quantity of Phytin in rice was estimated as follows. A weighed quantity of rice was reduced to coarse powder shaken with 0.3% Hydrochloric Acid and then filtered. The residue

EXPERIMENT No. 47.—White Polished Rice + Polishings freed from substances soluble in 0.3% HCl.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	Red cock	1235	1165	1115	1032	
2	Brown hen	980	960	885	850	
3	Red cock	1940	1770	1635	1465	
4	Black hen	1285	1230	1140	1050	
5	Red cock	1405	1200	1070	915	Polyneuritis
6	Black cock	1560	1460	1440	1285	Polyneuritis
7	Black hen	1520	1415	1340	1230	Polyneuritis
8	Brown hen	1350	1145	1090	1022	Polyneuritis
9	Grey hen	1375	1240	1135	1055	Polyneuritis
10	White hen	1510	1450	1310	1225	
11	Red cock	1705	1645	1515	1440	Polyneuritis
12	Black hen	1400	1235	1140	1050	

Experiment No. 48.

White Polished Rice washed + Polishings (0.3% HCl insoluble fraction)

No.	Description.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	—
1	Black hen	1360	1325	1215	1140		
2	White cock	1255	1150	1060	Polyneuritis		
3	Red cock	1860	1775	1645	1695	Polyneuritis	
4	Brown hen	1295	1220	1210	1170		
5	Red cock	1450	1335	1145	Died		No degeneration of nerve fibres.
6	Black cock	1530	1520	1435	1480	Polyneuritis	
7	Brown hen	1305	1270	1145	1080		
8	White hen	1665	1605	1500	1390		
9	Brown hen	1035	1040	985	920		
10	Red cock	1380	1390	1300	1185	Polyneuritis	
11	Black hen	1235	1215	1145	1080		
12	White cock	1340	1335	1280	Polyneuritis		

EXPERIMENT No. 49.—White Polished Rice + Polishings (0.3% HCl. soluble fraction).

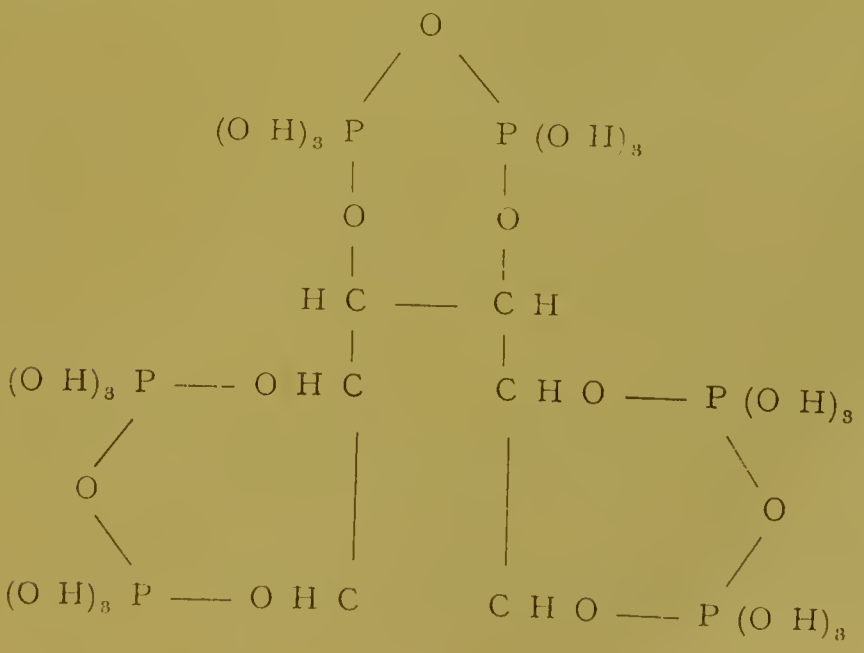
No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.	
1	Black hen	940	1000	1010	1080	1125	1000	
2	Black and brown cock ..	1440	1545	1615	1645	1665	1620	
3	Yellow cock	1520	1600	Died.				No degeneration of nerves.
4	Black hen	1285	1390	1360	1300	1320	1305	
5	Brown hen	1110	1195	1255	1240	1135	1160	
6	Yellow cock	1680	1775	1840	1915	1895	1750	
7	Brown hen	1185	1125	1055	1060	1035	1060	
8	Brown hen	1425	1445	1515	1540	1485	1520	
9	Brown cock	1305	1275	1390	1475	1480	1460	
10	Yellow hen	1010	1160	1265	1230	1190	1200	
11	Yellow hen	1170	1135	1155	1105	1145	1160	
12	Yellow hen	1315	1290	1330	1230	1180	1160	

was again shaken with acidulated water, this process being repeated until the filtrate ceased to give a precipitate with either Copper Acetate or Alcohol. To the combined filtrates excess of Copper Acetate was added. The mixture was allowed to stand overnight, thereafter the precipitate was collected, washed, dried, and weighed. From that weight, on the assumption that each molecule of the Copper phytinate contained two atoms of Copper, the amount of Phytin was calculated.

It is admitted that the process is not strictly speaking an accurate one but it was the best available and was satisfactory for purposes of comparison between various kinds of rice and their content of this highly phosphorized compound.

The unpolished rice in use was by this method shown to contain 1.07% of Phytin, the same rice after polishing yielded only a trace and the polished rice after washing in water contained none.

The structural formula for Phytic Acid as given by Neuberg is more complex than that of Posternak and has three times the molecular weight.



Phytin for experimental purposes was prepared by the following method. Sifted polishings were mixed with 0.3 per cent. Hydrochloric Acid in the proportion of 300 grms. of the former to 2000 cc. of the latter, the mixture was stirred throughout the day and on the following morning was filtered through a Buchner's filter. The clear yellowish filtrate was mixed with one and a half times its volume of 95 per cent. Alcohol which produced a white precipitate of Phytin; the mixture was allowed to stand for a few days. The precipitate was collected washed with strong alcohol to free it from acid and dried in a vacuum dessicator. A friable cake of Phytin was obtained readily reducible to a white powder, soluble in water, yielding an opalescent solution with an acid reaction and giving on addition of Sodium Carbonate a white flocculent precipitate.

As shown on page 74, 100 grammes of sifted polishings yield an average of 8.47 grammes Phytin. Unpolished rice loses 10% of its weight on polishing and on this basis the percentage of Phytin in unpolished rice would be not less than 0.847%. In our experiments to test the value of Phytin it was assumed that unpolished rice contained 1.07% of that substance and thereby if an error did exist it was in favour of the Phytin.

The Phytin prepared by us contained 34.8% of Phosphorus Pentoxide, whereas according to the formula of Posternak it should have contained 47.6% we proved the presence of Protein in the Phytin and we considered that our preparation contained 73% of the Calcium Magnesium salt of an acid having the formula given by Posternak.

A fowl consuming 60 grms. of unpolished rice daily would be receiving 0.64 grms. of Phytin. A fowl receiving the same amount of washed polished and therefore Phytin-free rice would require to have in addition that amount of Phytin daily in order to bring the value of this diet in respect of Phytin up to that of an unpolished rice diet.

Two experiments were carried out with this compound. In the first (No. 50) twelve fowls received washed polished rice and in addition Phytin, which was given in the following manner; 9 grms. of Phytin were dissolved in distilled water, the solution neutralized with Sodium Carbonate and the volume made up to 360 cc. Each fowl received 15 cc. of this suspension at 10.30 a.m. and 3.30 p.m. daily. All the fowls lost weight and cases of polyneuritis occurred just as if the fowls had received washed polished rice only.

In this experiment the fowls had the phosphorus pentoxide content of their diet augmented to 0.69% an amount in excess of that contained in a diet of unpolished rice but which on the assumption that our Phytin was only of 73% purity meant that they received daily only 0.54 grms. of Phytin in place of 0.64 grm.

In the next experiment (No. 51) twelve fowls received daily 1 grm. of Phytin which was prepared as an emulsion and intimately mixed with the washed polished rice but the results were the same.

Again assuming that our Phytin was only of 73% purity each fowl received daily 0.73 grm. of Phytin an amount sufficiently in excess of the calculated amount and certainly considerably in excess in respect of Phosphorus Pentoxide.

As shown subsequently the alcoholic filtrate from which the Phytin had been removed, freed from alcohol, was effective in protecting fowls on unpolished rice from the occurrence of polyneuritis. The importance of Phytin has therefore been disproved.

As the precipitate phytin had been shown to be ineffective, on theoretical grounds it was assumed that the whole of the active substances were contained in the filtrate.

The fraction remaining in solution after the precipitation of Phytin was next tested by the following procedure.

Polishings in quantities of 180 grms. were extracted with 0.3% Hydrochloric acid in the manner described on page 68 and the combined filtrates from each 180 grms. were mixed with one and a half times their volume of 95% alcohol. The precipitate was filtered off. A large number of weighings of this precipitate (Phytin) were made and it was found that an average of 8.47 grammes of Phytin were obtained from 100 grammes of sifted polishings.

The alcoholic filtrate was then nearly neutralised with Sodium Carbonate and evaporated at a low temperature until free from alcohol. The residue was diluted with distilled water to a volume of 1080 cc.. 30 cc. of this suspension contained the almost Phytin-free soluble substances from 5 grms. of polishings.

Two experiments were carried out with this solution.

In the first (Experiment 52) six fowls were fed on washed polished rice, each receiving daily 30 cc. of this suspension. All remained healthy.

In the second (No. 53) twelve fowls were employed with a similar result.

That fraction of the substances originally soluble in 0.3% HCl, which still remains in solution on the addition of alcohol, *the acid soluble, alcohol soluble part*, have thus been shown to contain the whole of the substances physiologically active, and an attempt was made to further divide this fraction by alkalinising.

An experiment carried out with the precipitate, *the acid soluble, alcohol soluble, alkali precipitatable part*, and another experiment with the filtrate, *the acid soluble, alcohol soluble, alkali solubie part*, had the unexpected result that the substances sought for were found to be no longer physiologically active in either fraction.

EXPERIMENT No. 50.—White Polished Rice + Polishings (0.3% HCl soluble, Proof spirit insoluble, fraction).

No	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week
1	Brown hen	980	970	910	805
2	Red cock	2000	1895	1700	Polyneuritis
3	Red cock	1980	1920	1775	Polyneuritis
4	Brownish cock ..	1395	1360	1315	Polyneuritis
5	Brown hen	1790	1750	1670	1565
6	Brown hen	1070	1050	980	865
7	White hen	1235	1240	1135	1025
8	Red hen	1205	1210	1170	1060
9	Brown hen	1175	1000	885	770
10	Grey hen	1150	1095	975	890
11	Yellow hen	1150	1015	980	930
12	Red hen	970	905	810	750

EXPERIMENT No. 51.—White Polished Rice + Polishings (0.3% HCl soluble, Proof spirit insoluble, fraction.)

No.	DESCRIPTION	Original wt.	1st week.	2nd week.	3rd week.
1	Red and Yellow cock	1420	1370	1305	1150
2	Red cock	1310	1280	1207	Polyneuritis
3	Red and black cock ..	1180	1150	1140	Polyneuritis
4	Yellow hen	1040	990	910	815
5	Black and red cock ..	1340	1360	1290	Polyneuritis
6	Yellow hen	1240	1225	1160	1065
7	Black hen	940	995	970	960
8	Yellow and black cock	1285	1320	1157	1005
9	Brown hen	1380	1375	1335	1200
10	Red and black cock ..	1430	1420	1355	1215
11	Yellow hen	995	945	905	815
12	Brown and black cock	1050	1060	1005	Polyneuritis

EXPERIMENT No. 52.—White Polished Rice + Polishings (0.3% HCl soluble, Proof spirit soluble, fraction.)

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	Red cock	2095	2140	2100	2060	2050
2	Red cock	1265	1280	1380	1275	1265
3	Brown hen	1550	1695	1655	1625	1660
4	Brown hen	900	850	905	915	935
5	Brown hen	1410	1430	1425	1445	1460
6	Black hen	1105	1160	1075	1070	1100

EXPERIMENT No. 53.—White Polished Rice + Polishings (0.3% HCl soluble, Proof spirit soluble, fraction).

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week
1	Yellow cock	1248	1267	1310	1310	1215
2	Speckled cock ..	1330	1425	1450	1525	1560
3	Red cock	1590	1645	1650	1635	1605
4	Yellow cock	1350	1305	1325	1325	1350
5	Red cock	1557	1535	1595	1585	1565
6	Black cock	1174	1240	1345	1385	1390
7	Red cock	1510	1510	1590	1615	1635
8	White cock	1530	1675	1690	1760	1805
9	Speckled cock ..	1467	1480	1535	1600	1610
10	Yellow cock	1507	1575	1605	1730	1785
11	White cock	1170	1215	1280	1270	1250
12	Red cock	1430	1400	1400	1370	1390

Experiments with the Proof Spirit Filtrate.

Following on the demonstration that the effective substances sought for were contained in the filtrate and therefore soluble in alcohol of proof spirit strength, experiments were undertaken with a view to isolating and testing the value of the various substances contained in that solution.

The solution was found to contain substances giving the reactions characteristic of proteins. On increasing the alcoholic strength of this liquid a precipitate was produced and it was hoped that by sufficiently increasing the amount of alcohol the protein would be precipitated.

By experiments in which the proof spirit filtrate was nearly neutralised by means of Sodium Carbonate, freed from alcohol by evaporation at a low temperature, and the alcohol-free filtrate treated with varying quantities of 95% alcohol, it was found that seven volumes of this alcohol produced a precipitate which was not appreciably less than that produced when eight volumes of 95% alcohol were added. The addition of seven volumes of alcohol produced a mixture containing 85% of alcohol, with six volumes it contained 71%, and with eight volumes the mixture contained 84% of alcohol. The increase of alcoholic strength being therefore only 1% when eight volumes were employed in place of seven volumes and the amount of precipitate not being appreciably increased, it was decided to carry out an experiment in which the alcohol-free filtrate was treated with seven times its volume of 95% alcohol. The following procedure was adopted.

The proof spirit filtrate obtained from 30 grammes of polishings and measuring from 650—660 cc. was placed in a glass evaporating basin and partially neutralized. In all previous experiments the partial neutralization of this liquid had been effected by means of a solution of Carbonate of Soda of unknown

strength, but in order to secure consistency in this and all subsequent experiments a normal solution of Carbonate of Soda was employed. By experiment it was found that 13 cc. of this solution were required for neutralization of the proof spirit filtrate from 30 grammes of polishings; 10.5 cc. left the liquid slightly acid and it was decided that this amount should be added to each quantity of proof spirit filtrate evaporated. The partially neutralized liquid was evaporated at a temperature of 60°C until free from alcohol.

To the alcohol-free liquid seven times its volume of 95% alcohol were added, the mixture stirred, allowed to stand for two days and then filtered. The precipitate weighed on an average 0.7 gramme and consisted partly of Phytin; it was freed from alcohol by exposure to the air and suspended in 180 cc. of distilled water. This volume contained the substances in 30 grammes of polishings soluble in 0.3% HCl, soluble in proof spirit and insoluble in alcohol of 83% strength.

In Experiment No. 54 each of six fowls on white polished rice received daily in addition 30 cc. of this suspension, cases of polyneuritis occurred.

The 83% alcoholic filtrate was freed from alcohol by evaporation at a temperature of 60°C and to the alcohol-free residue distilled water was added to make the volume up to 180 cc. This volume contained the substances in 30 grammes of polishings soluble in 0.3% HCl, soluble in proof spirit and soluble in 83% alcohol.

In Experiment No. 55 each of six fowls on white polished rice received daily in addition 30 cc. of this solution and cases of polyneuritis did not occur.

It was observed that when the 83% alcoholic filtrate stood a few days a further slight precipitate occurred and in the next experiment the alcohol free liquid mixed with seven volumes of 95% alcohol was allowed to stand ten days and then filtered.

EXPERIMENT No. 54.—White Polished Rice + Polishings (fraction soluble in 0.3% HCl, and in Proof spirit, and insoluble in 83% Alcohol.)

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week	5th week.
1	Spotted cock	1245	1300	1215	1100	Polyneuritis	
2	Black cock	1450	1490	1525	1540	1345	
3	Brown cock	1320	1350	1395	1405	1330	
4	White cock	1060	1145	1175	1105	1065	
5	Black cock	1290	1340	1375	1380	1325	
6	Brown cock	1510	1620	1665	1510	1315	

EXPERIMENT No. 55.—White Polished Rice + Polishings (fraction soluble in 0.3% HCl, soluble in Proof Spirit and soluble in 83% Alcohol.)

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	Red cock	1195	1245	1285	1255	1235
2	Red cock	1550	1525	1555	1560	1545
3	Yellow cock	1160	1160	1185	1135	1130
4	White cock	1120	1165	1200	1110	1125
5	Brown cock	1140	1185	1235	1145	1185
6	Red cock	1320	1315	1430	1440	1430

The precipitate and filtrate were treated as in the preceding experiments.

In Experiment No. 56 each of six fowls on white polished rice received in addition daily their proportion of the 83% alcohol —insoluble substances and cases of polyneuritis occurred.

In Experiment No. 57 each of six fowls on white polished rice received in addition daily their proportion of the 83% alcohol —soluble substances and cases of polyneuritis did not occur.

It was thus proved that the protective substances were soluble in 83% alcohol and were not inactivated by contact with alcohol of that strength for a period of ten days.

In order that the strength of the alcoholic mixture might be considerably increased and the volume of the mixture kept within working limits absolute alcohol in place of 95% alcohol was employed in the next series of experiments. The procedure adopted was as follows:—

The proof spirit filtrate obtained from the 0.3% HCl solution prepared from 30 grammes of polishings and measuring from 650—660 cc. was placed in an evaporating basin, 10.5 cc. of normal solution of Carbonate of Soda was added and the liquid evaporated at a temperature of 60°C until the volume was reduced to 50 cc. To this was added 600 cc. of absolute alcohol, the mixture stirred, allowed to stand for two days and then filtered. By this method the mixture produced was one containing 91% of Ethyl Alcohol.

The precipitate weighed on the average one gramme and was therefore appreciably greater in amount than that obtained from a mixture containing 83% of alcohol. It was freed from alcohol by exposure to the air and suspended in 180 cc. of distilled water. This volume contained the substances in

30 grammes of polishings soluble in 0.3% HCl, soluble in proof spirit, and insoluble in 91% alcohol.

In Experiment No. 58 each of six fowls on white polished rice received in addition daily 30 cc. of this suspension and cases of polyneuritis occurred.

The 91% alcoholic filtrate was evaporated at a temperature of 60°C until free of alcohol, the residue dissolved in distilled water and the volume adjusted to 180 cc. This volume contained the substances in 30 grammes of polishings, soluble in 0.3% HCl, soluble in proof spirit, and soluble in 91% alcohol.

In Experiment No. 59 each of six fowls on white polished rice received in addition daily 30 cc. of this yellowish turbid fluid and cases of polyneuritis did not occur.

It was thus shown that the protective substances are soluble in 91% alcohol.

EXPERIMENT No. 56.—White Polished Rice + Polishings (fraction soluble in 0.3% HCl, soluble in Proof spirit and insoluble in 83% alcohol).

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	Red cock	1245	1180	1040	Polyneuritis	
2	Brown cock	1425	1390	1320	1260	Polyneuritis
3	Speckled cock ..	1405	1440	1420	1275	Polyneuritis
4	White cock	1265	1165	1140	1080	Polyneuritis
5	Speckled cock ..	1435	1350	1290	1175	
	White cock	1080	950	890	800	

EXPERIMENT No. 57.—White Polished Rice + Polishings (fraction soluble in 0.3% HCl, soluble in Proof spirit, and soluble in 83% Alcohol).

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	White cock	1325	1390	1465	1425	1410
2	Black cock	1465	1390	1480	1420	1400
3	Black cock	1470	1460	1570	1480	1475
4	Yellow cock	1250	1120	1140	1155	1190
5	Black cock	1350	1335	1395	1335	1330
6	Red cock	1275	1180	1240	1230	1215

EXPERIMENT No. 58.—White polished rice + Polishings (fraction soluble in 0.3% HCl, soluble in Proof Spirit, and insoluble in 91% Alcohol).

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.
1	Red cock	1320	1250	1140	Polyneuritis
2	Red cock	1450	1340	1335	Polyneuritis
3	Speckled cock ..	1300	1285	1275	1285
4	Red cock	1420	1305	1185	Polyneuritis
5	Black cock	1495	1370	1430	1220
6	Black cock	1360	1290	1285	1130

EXPERIMENT No. 59.—White Polished Rice + Polishings (fraction soluble in 0.3% HCl, soluble in Proof spirit and soluble in 91% alcohol).

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	Red cock	1215	1285	1280	1285	1250
2	Speckled cock ..	1540	1600	1625	1630	1560
3	Red cock	1115	1190	1240	1270	1250
4	Grey cock	1250	1280	1360	1455	1435
5	Black cock	1550	1525	1675	1750	1775
6	Black cock	1415	1420	1570	1560	1535

Experiments with 91% Alcohol Filtrate.

Experiments were undertaken with a view to the isolation and testing of the various substances contained in the 91% alcoholic filtrate.

This filtrate was a clear yellowish liquid. In view of the fact that the protective substance or substances are destroyed by exposure to steam under pressure, it was considered probable that the activity of the liquid was not due to the presence of salts, but rather to the presence of some organic compound or compounds.

When the filtrate is freed from alcohol and the residue mixed with distilled water a yellowish turbid liquid is obtained. This liquid on saturation with Ammonium Sulphate yields a reddish brown precipitate which gives the usual protein reactions. Half saturation with Ammonium Sulphate produces a slight precipitate. Saturation with Sodium Chloride produces a precipitate and on filtration the filtrate saturated with Ammonium Sulphate gives a further precipitate.

As many vegetable globulins are not precipitated until their solutions are nearly saturated with Ammonium Sulphate it cannot be inferred that the alcoholic filtrate contains both a globulin and albumin.

Unpolished rice was tested and found to contain alcohol soluble proteins while polished rice similarly tested was found to contain none. It would appear therefore that Rosenheim and Kajuira were in error in stating that rice did not contain alcohol-soluble proteins. It seems probable that they did not examine unhusked rice (padi) and unpolished rice not being an article of commerce would of course not be available.

The alcoholic filtrate freed from alcohol and the residue solved in distilled water was tested with Fehling's Solution and gave a precipitate of Cuprous Oxide. The solution was heated with Phenylhydrazine Hydrochlorate and Sodium Acetate; crystals of Phenyl-glucosazone were obtained. Another portion of the liquid was saturated with Ammonium Sulphate and filtered, the filtrate was heated with Phenylhydrazine Hydrochlorate and Sodium Acetate and crystals of Phenyl-glucosazone were obtained.

The 91% alcoholic filtrate evaporated to dryness and the residue dried in a dessicator was found to contain, after making allowance for the Sodium Chloride formed on partial neutralization with normal solution of Sodium Carbonate, total solids amounting to 14.2 % of the sifted polishings. These solids form a brown, sticky, hygroscopic residue.

Gravimetrically it was determined that 2.9 grammes of glucose, and by the Kjeldahl process that 2.08 grammes of protein are contained in this 14.2 grammes of total solids. The remaining 9.22 grammes was assumed to be salts.

It was sought to isolate and test the value of the alcohol soluble proteins. For this purpose dialysis was tried but the conditions here are unsuitable for dialysis, as even with the addition of Thymol, a mass of moulds and bacteria develop in a day or two both in the dialyser and the water. Unfortunately we had not a porcelain filter suitable for attachment to the water supply and eventually dialysis for the purpose of separating the alcohol-soluble proteins had to be abandoned.

It was decided to employ Ammonium Sulphate for the separation of these bodies and the following procedure was adopted:—

The 91% alcoholic filtrate, representing the quantity obtained from 30 grammes of polishings, was evaporated at 60°C till free from alcohol; the residue was dissolved in distilled water and

the volume adjusted to 100 cc. To this solution was added 72 grammes of Ammonium Sulphate being the amount found by experiment to be necessary for saturation. The mixture was agitated until the salt was entirely dissolved, then filtered.

The reddish brown precipitate obtained was solved in distilled water and the volume adjusted to 180 cc. This volume contained the substances in 30 grammes of polishings, soluble in 0.3% HCl, soluble in proof spirit, soluble in 91% alcohol and precipitated by saturation of the latter solution freed from alcohol with Ammonium Sulphate. The yellowish fluid obtained was a solution of the alcohol-soluble proteins in a dilute solution of Ammonium Sulphate. The fact that the proteins were soluble in this fluid suggests that they were not denatured.

In experiment No. 60 each of six fowls on white polished rice received in addition daily 30 cc. of this solution. It was calculated experimentally that each fowl received daily not more than 0.5 gramme of Ammonium Sulphate. All the fowls lost weight and one case of polyneuritis showing marked degenerative nerve-changes occurred in the fourth week.

The filtrate, obtained after saturation of the protein containing solution, was useless for feeding experiments because it was saturated with Ammonium Sulphate. This filtrate after standing a few days showed a deposit of crystals which on examination were found to be Magnesium Sulphate.

This filtrate freed from proteins and assuming that it contained only sugar and salts ought to be completely dialysable.

It was thought probable that this process might more readily be carried out on the protein-free fluid; but even in the case of this fluid, after two days dialysis in running water, the outside of the parchment paper was covered with a slime of micro-organisms. The addition of Thymol to the contents of the dialyser prevented growths occurring inside the dialyser but Thymol in the water did not prevent the growth on the outside.

Dialysis was only possible by the following procedure. The protein-free filtrate was placed in the dialyser along with a piece of Thymol; after two days dialysis in running water, the fluid was heated on a water bath, the parchment paper renewed and the fluid dialysed for another two days. It was heated again, the paper renewed and a further two days dialysis carried out. It was then heated and dialysed into distilled water changed daily for six days. The fluid remaining after dialysis for twelve days was evaporated to dryness and a slight residue of a yellowish colour was obtained.

The residue weighed 0.02 gramme, it was soluble in water, and partly organic. It ammounted to not more than 0.02% of the solids contained in the fluid dialysed.

It was observed that the fowls fed on white rice *plus* the alcohol-soluble proteins lost weight and it was considered possible that part of these substances might have been lost in the course of various manipulations to which the fluid had been subjected. On account of the large quantities of absolute alcohol required it was not possible to prepare the alcohol soluble proteins in such quantities as to enable us to give each fowl daily the amount obtained from 10 grammes of polishings. It appeared simpler to employ the original proof spirit filtrate. It may here be remarked that the active substances are unchanged by contact for months with proof spirit. In this experiment the procedure adopted was as follows:—

The proof spirit filtrate in quantities representing the materials from 30 grammes of polishings dissolved out by 0.3% HCl, soluble in proof spirit and measuring usually from 650—660 cc. was placed in glass evaporating basins. To each lot 10.5 cc. of normal solution of Carbonate of Soda was added and evaporation carried out at 60°C.

The alcohol free fluid obtained from each lot was made up to 100 cc. with distilled water saturated with 72 grammes of Ammonium Sulphate and filtered.

The precipitate was dissolved in distilled water and the volume adjusted to 180 cc. This contained the substances in 30 grammes of polishings, dissolved out by 0.3% HCl, soluble in proof spirit, and precipitated by Ammonium Sulphate.

In Experiment No. 61 each of six fowls on white polished rice received in addition daily 30 cc. of this solution. At the end of one week all but one had lost weight and it was decided to give them an additional 30 cc. daily, the second dose being given half an hour after they had received the afternoon meal. Even with this increased amount the fowls continued to lose weight and at the close of the fourth week one case of what appeared to be polyneuritis occurred but examination of the nerves failed to reveal the presence of degenerative changes.

The experiment was continued for 31 days and on the thirtieth day another similar case occurred but again there were no degenerative changes in the nerves.

It must therefore be concluded that the alcohol-soluble proteins are not by themselves sufficient to protect fowls fed on white rice from the occurrence of polyneuritis. This conclusion is based on the assumption that the alcohol-soluble proteins were unchanged by the treatment to which they had been subjected.

In previous experiments it has been shown that the protective substances are extracted from parboiled rice by the use of hot 94% alcohol. It was thought possible that, as polishings are in a much finer state of sub-division, agitation with 95% alcohol might suffice to extract the protective substances and not the protein but even by this process both protein and glucose were extracted; a similar result was obtained with absolute alcohol. Experiments are now being undertaken to determine if the protective substance or substances can be separated from the Ammonium Sulphate filtrate or from polishings by the use of other solvents, as for example Ethyl acetate.

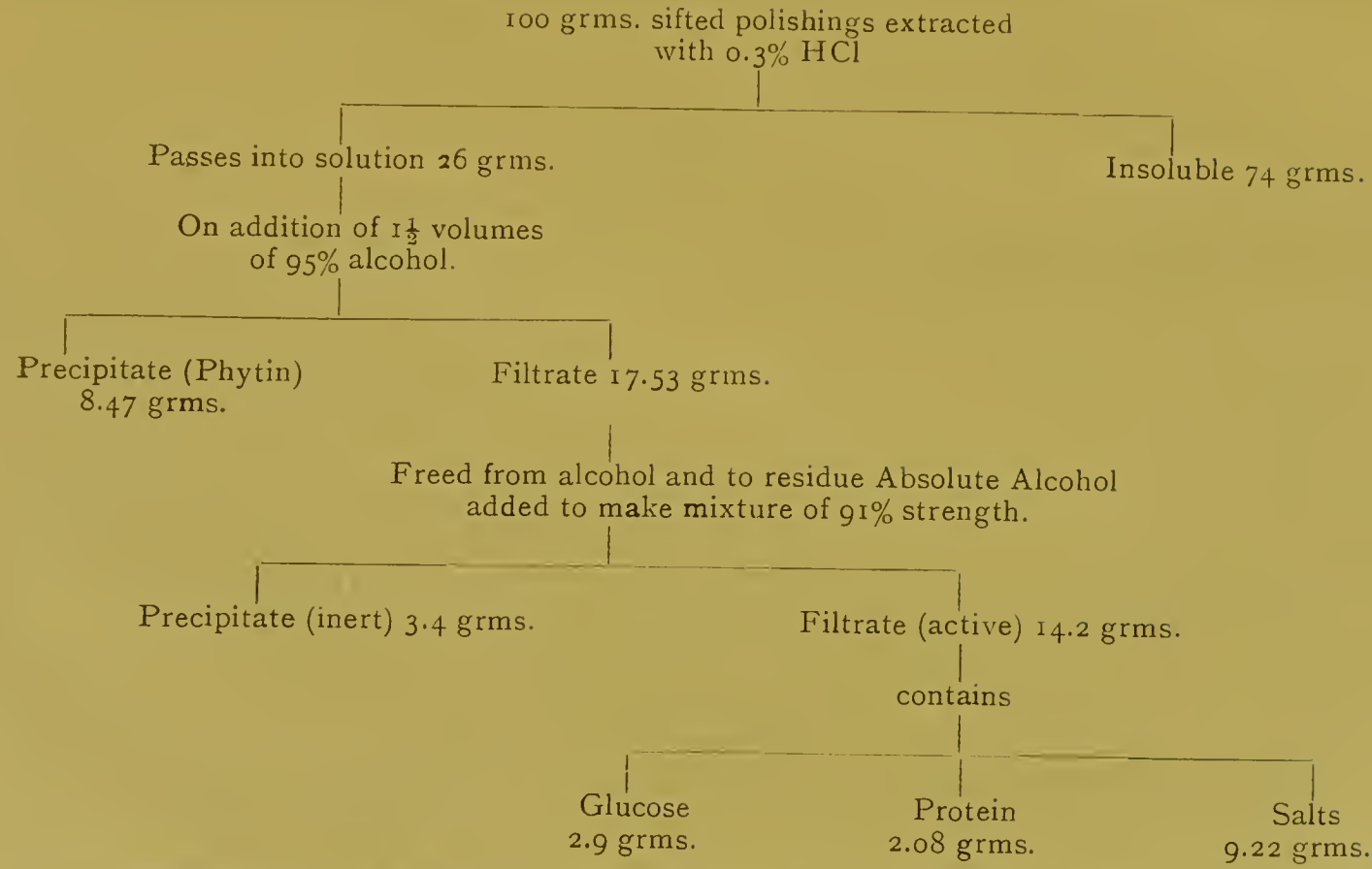
It may be that the 91% alcoholic filtrate contains substances other than Proteins, glucose, and salts, but until such time as the various constituents of that filtrate have been isolated, tested and identified, the biological reaction remains the only method by which the presence of the protective substances can be detected.

EXPERIMENT No. 60.—White Polished Rice + Ammonium Sulph. precipitate from 91% alcohol filtrate.

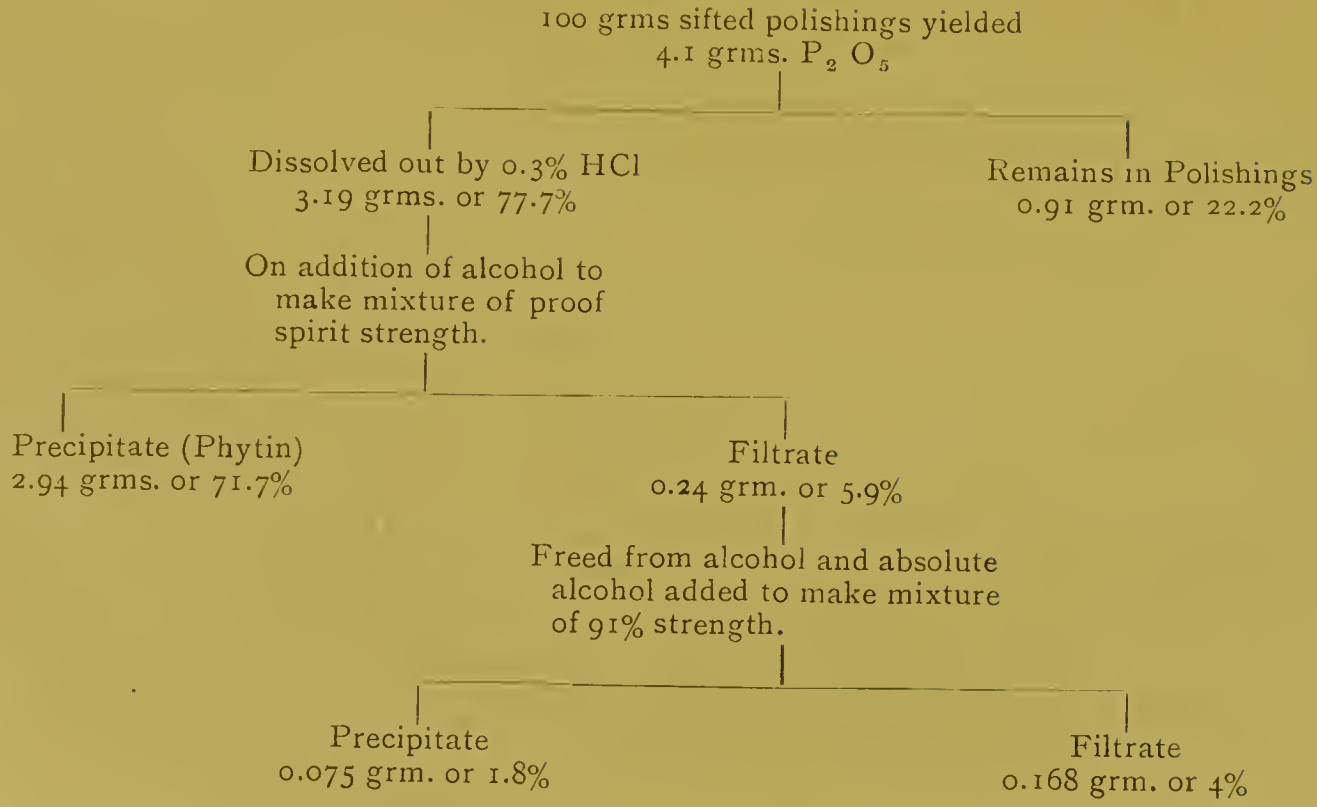
No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.
1	White cock	1185	1075	955	950	910
2	Brown cock	1100	985	955	960	955
3	Brown cock	1370	1330	1340	1285	1245
4	White cock	1115	1105	1035	1025	1000
5	Black cock	1180	1145	1120	1065	1025
6	Brown cock	1165	1040	1005	915	Polyneuritis

EXPERIMENT No. 61.—White Polished Rice + Ammon. Sulph. precipitate from Proof Spirit Filtrate.

No.	DESCRIPTION.	Original wt.	1st week.	2nd week.	3rd week.	4th week.	5th week.
1	Red cock	1070	1040	1020	930	840	
2	White cock	1020	1030	1015	965	Polyneuritis	
3	Brown cock	1135	1090	980	895	810	
4	Brown cock	1270	1195	1080	970	785	
5	Black cock	1455	1415	1330	1230	1200	
6	Brown cock	1115	1025	1015	910	800	



NOTE.—These figures are based on an average of all observations and include many made since the publication of a paper in the *Lancet* No. 4555 of 17th December, 1910. They differ to a slight extent from those recorded in that paper.


100 grms sifted polishings yielded
4.1 grms. $P_2 O_5$
Dissolved out by 0.3% HCl
3.19 grms. or 77.7%
Remains in Polishings
0.91 grm. or 22.2%
On addition of alcohol to make mixture of proof spirit strength.
Precipitate (Phytin)
2.94 grms. or 71.7%
Filtrate
0.24 grm. or 5.9%
Freed from alcohol and absolute alcohol added to make mixture of 91% strength.
Precipitate
0.075 grm. or 1.8%
Filtrate
0.168 grm. or 4%

	Effect on fowls; estimated by the occurrence of polyneuritis.	Estimated amount in grms. of fat in diet.	Estimated amount in grms. of P_2O_5 in diet.
Unpolished rice	−	0.99	0.336
Polished rice plus polishings	−	0.83	0.357
Polished rice	+	0.13	0.156
Washed polished rice	+	0.13	0.126
Washed polished rice plus fat-free polishings	−	0.16	0.326
Washed polished rice plus polishings	−	0.83	0.331
Polishings extracted with 0.3% HCl.			
Washed polished rice plus extracted polishings	+	0.83	0.165
Washed polished rice plus extract	−	0.13	0.281
Extract mixed with one and a half times its volume of 95% Alcohol.			
Washed polished rice plus precipitate	+	0.13	0.27
Washed polished rice plus filtrate	−	0.13	0.138
Filtrate freed from alcohol and absolute alcohol added to residue to make mixture of 91% strength.			
Washed polished rice plus precipitate	+	0.13	0.129
Washed polished rice plus filtrate	−	0.13	0.133

Conclusions.

1. The occurrence of Beri-beri in the Malay Peninsula has an intimate relationship with the consumption of a diet of which white polished rice forms the staple. Those who consume unpolished rice or slightly polished (native or Malay, or parboiled) rice do not suffer from the disease.

2. Fowls fed on white polished rice known to have been associated with outbreaks of human beri-beri develop a form of polyneuritis clearly analagous to Beri-beri in its clinical manifestations and pathological effects. Other white polished rices produce a similar result. Fowls fed on unpolished rice remain healthy.

These animals may therefore be employed to study the mode of operation by which a diet of white polished rice results in Beri-beri in man.

3. The estimation in terms of phosphorus pentoxide of the total phosphorus content of a given rice may be used as an indicator of the extent to which such a rice has been milled or polished and therefore of its Beri-beri producing power when forming the staple of a diet in man.

4. The harmful influence of white polished rice is not due to the existence in it of a poison developed after milling. White polished rice makes default in respect of some substance of high physiological importance essential for the maintenance of health.

5. Fowls fed on white polished rice constantly develop polyneuritis in a period of three to four weeks.

6. If the meal or polishings removed from such white rice in the process of milling be added to a diet of white polished rice, fowls remain healthy.

Substances essential for the maintenance of health are therefore contained in polishings.

7. Unpolished rice which has been submitted to sterilization in the autoclave at a temperature 120°C for two hours will cause polyneuritis when fed to fowls. The protective substances are destroyed under these conditions.

Methods of analysis involving exposure to high temperatures are therefore unsuitable for determining the nature of the protective substances.

8. The fats contained in the peripheral layers of the grain are of no value in protecting against polyneuritis.

9. The protective substances are soluble in 0.3% Hydrochloric Acid.

Phytin which comprises 32.5% of the substances so soluble is without value as a protective.

10. The substances are not precipitated from solution in 0.3% Hydrochloric Acid on the addition of 95% alcohol in such quantity as to make the resulting mixture of proof spirit strength.

They are soluble in proof spirit containing approximately 0.12% Hydrochloric Acid.

11. The protective substances are soluble in a slightly acidulated solution containing 91% of alcohol and, exclusive of glucose, amount to not more than 11.3% by weight of rice polishings and not more than 1.13% of the original unpolished rice grain. In this fraction are included prolamine (alcohol soluble protein) and compounds of calcium, magnesium and phosphorus.

These researches, which comprise an unbroken sequence of experiments beginning with rices associated with outbreaks of human beri-beri, demonstrate that rice is rendered harmful by the milling and polishing process to which it is subjected in

the preparation of white polished rice. In this process there is removed from the grain some substance of high physiological importance in the metabolism, the absence of which results in the production of polyneuritis in fowls and of beri-beri in man when a diet is consumed of which white polished rice is the staple. Whether these substances act by rendering other elements in the diet available for nutrition or whether they are themselves the nutritive material necessary for nerve tissues can in our present state of knowledge only be matter for conjecture. These substances, small in amount as compared with the total of the diet, have been determined within certain narrow limits but their exact chemical nature is still unknown.

There is no evidence that white rice contains a poison generated after decortication by the action of moulds or other organisms.

As measures for the prevention of beri-beri in this country it is recommended that the use of unpolished or under-milled rice be encouraged among those classes of the community in which the disease occurs. The polishing process if carried out at all should not extend beyond the removal of the outer skin or pericarp. The parboiling of rice before milling, as recommended by Dr. Braddon, serves the important purpose of so hardening the outer layers of the grain that their removal is less easy and over-milling is less likely to occur. The cooking of rice by steam under pressure should be prohibited. As an indicator of the extent to which rice has been milled we recommend to chemists the use of the phosphorus pentoxide standard. In the examination of a large number of rices, none were found associated with human beri-beri or polyneuritis in fowls which yielded a phosphorus pentoxide content of 0.4% or over, as estimated on the undried material. The amount of moisture varied only slightly and none of the rices were faced.

REFERENCES.

(1). Wernich.—"Geographisch-medicinische Studien," Berlin, 1878.

(2). Van Leent.—"*Geneesk Tijdschr, voor Nederl-Indie*," 1880.

(3). Takaki.—"Prevention of Kakke," in Japanese Navy, *Sei-i-Kwai*, 1885, 1886, 1887.

(4). Idem.—"The Health of the Japanese Navy and Army," *Lancet*, 1906, Nos. 1369, 1451, 1520.

(5). Durham, H. E.—"Notes on Beri-beri in the Malay Peninsula and on Christmas Island," *Journal of Hygiene*, 1904, Vol. IV, No. 1.

(6). Braddon, W. L.—"The Etiology of Beri-beri," *F. M. S. Medical Archives*, 1901.

"The Cause and Prevention of Beri-beri," London, 1907.

(7). Fletcher, W.—"Rice and Beri-beri," *Lancet*, 1907, Vol. 1, p. 1776; "Rice and Beri-beri," *Journal of Tropical Medicine and Hygiene*, 1909, No. 12, p. 127.

(8). Fraser, H. and Stanton, A. T.—"An Inquiry Concerning the Etiology of Beri-beri," *Lancet*, 1909, No. 4459, p. 451.

"An Inquiry Concerning the Etiology of Beri-beri," Studies from the Intitute for Medical Research, 1909, No. 10.

(9). Schaumann, H.—"Die Atiologie der Beri-beri unter Berucksichtigung des gesamtem Phosphorstoffwechsels," Leipzig, 1910.

(10). Hooper, D.—"The Composition of Indian Rice," *The Agricultural Ledger*, 1909, No. 5, Calcutta.

(11). Watt.—"The Commercial Products of India," London, 1908.

(12). Eijkman, C.—"Eine Beri-beri-ähnliche Krankheit der Hühner," *Vichows Archiv*, 1897.

"Polyneuritis bei hoenders, nieuwe tot de aetiologie der *Ziekte*," *Geneesk. Tijdschr. v. Neederl. Indie*, 1896.

(13). Grijns, G.—"Over Polyneuritis gallinarum I, II, III," *Geneesk. Tijdschr. voor Nederll Indie*, 1901, 1909, 1910.

(14). Hulshoff Pol, D. J.—"Beri-beri. Voorkoming en genezing door toediening van Katjang hidjoe," Amsterdam, 1904.

"Beri-beri en Katjang-hidjoe," *Geneesk. Tijdschr. voor Nederl. Indie*, 1906.

"X—Zuur, het tegen Beri-beri werkzame bestanddeel uit de Katjang hidjoe," *Geneesk. Tijdschr. voor Nederl. Indie*, 1907.

(15). Kiewiet de Jonge, G. W.—"Onderzoekingen over Beri-beri," *Geneesk. Tijdschr. voor Nederl. Indie*, 1909.

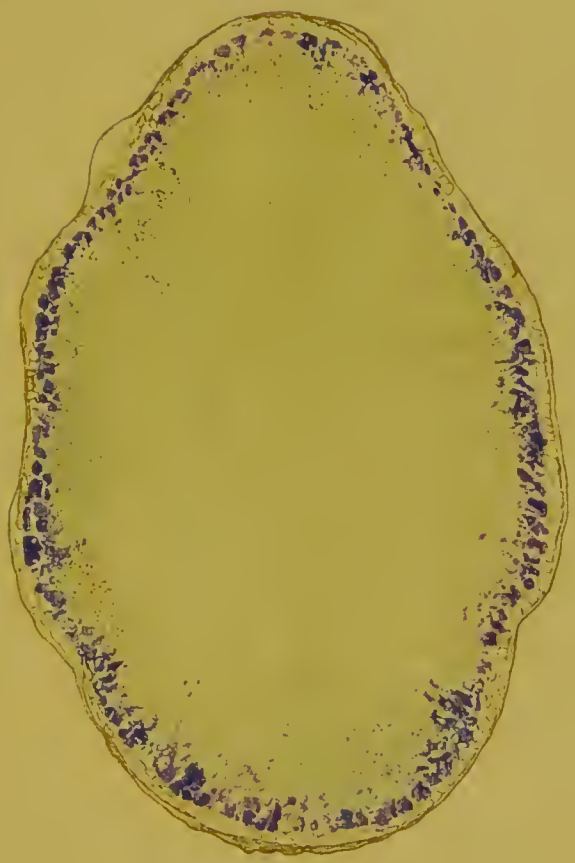
(16). Wright, H.—"Studies from the Institute for Medical Research," Vol. II, No. 1, 1902.

(17). Daniels, C. W.—"Studies from the Institute for Medical Research," Vol. IV, Pt. 1, 1906.

(18). Holst, A. and Fröhlich, T.—"Experimental Studies relating to Ship Beri-beri and Scurvy," *Journal of Hygiene*, 1907, Vol. VII, No. 5.

(16). Aron, H.—"Phosphorus Starvation with special reference to Beri-beri," *Philippine Journal of Science*, 1910, Vol. V, No. 1.

PLATE I



TRANSVERSE SECTION OF PADI (HUSK REMOVED)

STUDIES

FROM

THE INSTITUTE FOR MEDICAL RESEARCH,

FEDERATED MALAY STATES.

No. 1. Vol. 1.—No. 1. The Malarial Fevers of British Malaya by HAMILTON WRIGHT, M.D. (McGILL).

No. 2. Vol. 2.—No. 1. An Inquiry into the Etiology and Pathology of Beri-Beri by HAMILTON WRIGHT, M.D. (McGILL).

No. 3. Vol. 2.—No. 2. On the Classification and Pathology of Beri-Beri by HAMILTON WRIGHT, M.D. (McGILL).

No. 4. Vol. 3.—Part 1. The Diseases of British Malaya by C. W. DANIELS, M.B. CAMB.

No. 5. Vol. 3.—Part 2. Water Supplies (Preliminary Observations) by C. W. DANIELS, M.B. CAMB.

No. 6. Vol. 3.—Part 3. Breeding Grounds of Culicidæ by C. W. DANIELS, M.B. CAMB. The Culicidæ of Malaya by G. F. LEICESTER, M.B., C.M. EDIN.

No. 7. Vol. 3.—Part 4. The Outbreaks of Rinderpest in Selangor, 1903 and 1904, by C. W. DANIELS, M.B. CAMB.

No. 8. Vol. 4.—Observations on Beri-Beri by C. W. DANIELS, M.B. CAMB.

No. 9. Surra in the Federated Malay States by HENRY FRASER, M.D. (ABER.) and S. L. SYMONDS, V.S. (MELB.).

No. 10. An Inquiry concerning the Etiology of Beri-Beri by HENRY FRASER, M.D. (ABER.) and A. T. STANTON, M.D. (TOR).

No. 11. The Etiology of Beri-Beri by HENRY FRASER, M.D. (ABER.) and A. T. STANTON, M.D. (TOR).

No. 12. The Etiology of Beri-Beri by HENRY FRASER, M.D. (ABER.) and A. T. STANTON, M.D. (TOR).